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H. W. WILEY, Chief of Bureau.

PROCEEDINGS

OF THE

TWENTY-SIXTH ANNUAL CONVENTION

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS,

HELD AT

DENVER, COLORADO, AUGUST 26-28, 1909.

EDITED BY

HARVEY W. WILEY,
SECRETARY OF THE ASSOCIATION.

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LETTER OF TRANSMITTAL

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., January 28, 1910.

Sir: I have the honor to submit for your approval the Proceedings of the Twenty-sixth Annual Convention of the Association of Official Agricultural Chemists. All general discussion has been eliminated in view of the constantly increasing volume of the work, only the reports and papers being presented which are necessary to the conduct of the work, the improvement of methods of analysis, and the elaboration of new methods. I recommend that these Proceedings be published as Bulletin 132 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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FIRST DAY.

THURSDAY—MORNING SESSION.

By vote of the executive committee, Denver, Colo., was chosen as the meeting place of the twenty-sixth annual convention of the Association of Official Agricultural Chemists. The convention was called to order on the morning of August 26 at the Brown Palace Hotel, with Mr. W. D. Bigelow, of Washington, D. C., the president, in the chair. The following visitors and members were present:

MEMBERS AND VISITORS PRESENT.

Abbott, J. S., State Dairy and Food Commission, Denton, Tex.
Alford, F. C., Fort Collins, Colo.
Allen, W. M., Department of Agriculture, Raleigh, N. C.
Aschman, Frederic T., Department of Agriculture, Pittsburg, Pa.
Averitt, S. D., Agricultural Experiment Station, Lexington, Ky.

Bailey, E. H. S., State Board of Agriculture and State Board of Health, Lawrence, Kans.
Balcom, R. Wilfred, Food and Drug Inspection Laboratory, Nashville, Tenn.
Barnard, H. E., State Food and Dairy Commission, Indianapolis, Ind.
Bartlett, James M., Agricultural Experiment Station, Orono, Me.
Bigelow, W. D., Bureau of Chemistry, Washington, D. C.
Brown, Lucius Polk, State Food and Drug Laboratory, Nashville, Tenn.
Bryan, T. J., State Laboratory, Chicago, Ill.

Carpenter, Frank B., Virginia-Carolina Chemical Company, Richmond, Va.
Cathcart, Charles S., Agricultural Experiment Station, New Brunswick, N. J.
Chittick, J. R., Food and Dairy Commission, Des Moines, Iowa.
Cook, Alfred N., State Food Commission, Vermilion, S. Dak.

De Barr, Edwin, State Food Commission, Norman, Okla.
Dinsmore, Sanford C., Agricultural Experiment Station, Reno, Nev.
Dodson, William Rufus, Agricultural Experiment Station, Baton Rouge, La.
Dorset, Marion, Bureau of Animal Industry, Washington, D. C.

Eaton, Edward N., State Board of Agriculture, Chicago, Ill.
Ellett, Walter B., Agricultural Experiment Station, Blacksburg, Va.

Fischer, Richard, Dairy and Food Commission, Madison, Wis.
Fitz-Randolph, R. B., State Board of Health, Trenton, N. J.
Fraps, G. S., Agricultural Experiment Station, College Station, Tex.
Frear, William, Agricultural Experiment Station, State College, Pa.

Gudeman, Edward, Chicago, Ill.

Halligan, J. E., Agricultural Experiment Station, Baton Rouge, La.
Hand, William Flowers, State Laboratory, Agricultural College, Miss.
Harms, Herman, Dairy and Food Commission, Salt Lake City, Utah.
Hartwell, Burt L., Agricultural Experiment Station, Kingston, R. I.
Hill, Edward C., State Board of Health, Denver, Colo.
Hiltner, R. S., Food and Drug Inspection Laboratory, Denver, Colo.
Hoagland, Ralph, Bureau of Animal Industry, Chicago, Ill.
Horne, William Dodge, Yonkers, N. Y.
Hortvet, Julius, State Dairy and Food Department, St. Paul, Minn.
Huston, Henry A., Chicago, Ill.

Jaffa, Myer Edward, University of California, Berkeley, Cal.
Jones, Hamilton P., State Food Commission, New Orleans, La.

Kebler, L. F., Bureau of Chemistry, Washington, D. C.
Kellogg, James W., Department of Agriculture, Harrisburg, Pa.
Knight, Henry G., Agricultural Experiment Station, Laramie, Wyo.

Ladd, Edwin F., Agricultural Experiment Station, Fargo, N. Dak.
Leach, Albert E., Food and Drug Inspection Laboratory, Denver, Colo.
Lowenstein, Arthur, Chicago, Ill.
Lythgoe, Hermann C., State Board of Health, Boston, Mass.

Magruder, E. W., Department of Agriculture, Richmond, Va.
Mason, Glen F., Pittsburg, Pa.
Moudy, Ross B., Dairy, Food, and Oil Commission, Laramie, Wyo.
Munn, Carl S., Chicago, Ill.

Patten, Andrew J., Agricultural Experiment Station, East Lansing, Mich.

Read, M. T., Food and Drug Inspection Laboratory, Denver, Colo.
Redfern, Ellsworth L., Food, Dairy, and Drug Commission, Lincoln, Nebr.
Robison, Floyd W., State Dairy and Food Department, Lansing, Mich.
Rose, Rufus E., Department of Agriculture, Tallahassee, Fla.
Ross, B. B., state chemist, Auburn, Ala.
Ross, Walter M., city chemist, Kansas City, Mo.
Rudnick, Paul, Armour & Co., Chicago, Ill.

Shedd, O. M., Agricultural Experiment Station, Lexington, Ky.
Shilstone, Herbert M., Louisiana Sugar and Rice Exchange, New Orleans, La.
Smith, William Bradford, Bureau of Animal Industry, South Omaha, Nebr.
Speare, Howell D., Agricultural Experiment Station, Lexington, Ky.
Stallings, R. E., state chemist, Atlanta, Ga.
Street, John P., Agricultural Experiment Station, New Haven, Conn.

Traphagen, F. W., Denver, Colo.
Trowbridge, P. F., Agricultural Experiment Station, Columbia, Mo.

Weber, H. A., Ohio State University, Columbus, Ohio.
White, Horace L., Agricultural Experiment Station, Fargo, N. Dak.
Wiley, H. W., Bureau of Chemistry, Washington, D. C.
Willard, Julius T., Agricultural Experiment Station, Manhattan, Kans.
Wise, Frank H., Food and Drug Inspection Laboratory, Denver, Colo.
Withers, W. A., Agricultural Experiment Station, Raleigh, N. C.
Woll, Fritz Wilhelm, Agricultural Experiment Station, Madison, Wis.
Woods, Charles D., Agricultural Experiment Station, Orono, Me.

REPORT ON PHOSPHORIC ACID.

By W. F. Hand, *Referee.*

Introductory Discussion.

Suggestions to the referee on phosphoric acid for 1909 concerning the studies considered by the association of more immediate importance are outlined in the recommendations made at the last meeting in Washington, as follows:

(1) That the recommendation of 1907 be repeated, viz, that the referee on phosphoric acid shall take up for report at the next meeting of the association methods applicable under American conditions to the official examination of basic slag phosphates.

(2) That the referee make a further study of methods for the preparation of neutral ammonium citrate solution.

(3) That the referee investigate the amount of wash water to be employed in the treatment of the residue from the ammonium citrate digestion.

(4) That the above recommendation be amended to include a study of the manner of filtering.

BASIC SLAG PHOSPHATES.

The valuation of slag phosphates has been before the association for some time, and the limitations of the method based on fineness alone are well recognized. But before a satisfactory laboratory process for estimating the comparative availability under American conditions of phosphoric acid in these slags can be adopted a great deal of cooperative work must be done, and much time will be required necessarily in carrying out field experiments throughout the country and in laboratory studies in connection with the effect of slags on various soil types.

It appears, therefore, that this is a broad problem, which can not be studied in the most successful way by the referee on phosphoric acid from year to year. The general cooperation of the experiment stations should be secured, and as many members of this association are experiment-station officers there should be no great difficulty in enlisting their assistance, since this is a matter of growing importance and one in which many of the stations are already interested.

In view, therefore, of the circumstances, the referee believes that a special committee on basic slags should be appointed. This committee should make every effort to secure the assistance of the stations to work out a definite plan of procedure, and to institute as soon as practicable field tests to extend over a period of at least five years. The committee should make annual reports to the association of the progress of the work, and after sufficient data have been accumulated, a full report with appropriate recommendations can be brought before the association for consideration.

A careful and systematic study of this question from the standpoint of the American farmer will extend over a period of years, and, meanwhile, the present provisional method of estimating the comparative commercial value on the basis of total phosphoric acid and the fineness of the product affords little protection to purchasers and manufacturers of basic slags against adulteration of the genuine product with ground phosphate rock itself, or with inferior slags prepared by special methods from such rocks. The possibility of adulteration of this kind should be avoided, and, since the fineness method will not answer, the referee believes that the association should consider the provisional use of the well-known Wagner method, which has been subjected to much study abroad

and to some extent also in this country. The careful consideration of this matter is important, and the referee hopes that it will receive full attention at this meeting.

UNIFORMITY OF PROCEDURE IN THE DETERMINATION OF CITRATE-INSOLUBLE PHOSPHORIC ACID.

While the present official methods of preparing solutions, and also the procedure for the determination of citrate-insoluble phosphoric acid, have been in use for some time, the not infrequent variations in the results of analysts appear to emphasize the desirability of a method of preparing an official neutral ammonium citrate solution which will at least obviate to some extent the difficulties of the methods now in use, and which can be relied upon to result in a uniform reagent, and also the necessity for more minutely prescribing all of the details of the process of estimating insoluble phosphoric acid when a satisfactory method of securing an accurately neutralized ammonium citrate solution has been discovered.

But before proceeding with the study of this subject, it was thought best to ascertain if possible the degree of uniformity which prevails in carrying out the details of the present process, because it is evident from a consideration of the determination itself that uniformity in essential details is absolutely necessary for accordant results. With this object in mind, a large number of official chemists were requested to outline the procedure in their laboratories as follows:

(1) Details of method used in preparing the official neutral ammonium citrate solution.
(2) The kind and size of flask used in the ammonium citrate digestion.
(3) Depth to which the flask is usually immersed in the water bath.
(4) Average temperature of bath necessary to maintain solution in the flask at 65° C.
(5) Method of performing filtration.
(6) Size and kind of filter paper used.
(7) Efficiency and rapidity of filtration in the analysis of acid phosphates and of mixed fertilizers.
(8) Average total volume of filtrates after complete washing of the residue.
(9) Procedure in case filtrates are not clear.

Replies were received from 21 analysts, and these bring out in a striking way the lack of uniformity in the determination of insoluble phosphoric acid by the present official method.

METHODS OF PREPARING NEUTRAL AMMONIUM CITRATE SOLUTION.

The official method prescribes that the neutrality of the citrate solution be determined by the use of corallin as an indicator or by the removal of the citric acid by alcoholic calcium chlorid solution and testing finally with cochineal, as suggested by Houston. Replies to 21 inquiries showed that only 7 analysts relied upon corallin for the determination of the point of neutrality, though differing somewhat in detail in carrying out the neutralization. One used corallin, but always checked the results by litmus, or employed litmus but checked by corallin; one preferred cochineal; two relied upon the alcoholic calcium chlorid method, while eight made use of litmus solution, azolitmin or litmus paper, with modifications as to details. It should be noted also in this connection that one analyst used a solution of a specific gravity of 1.091 instead of 1.09, allowing thereby for the water in the washed sample.

These statements are sufficient to emphasize the desirability of a more careful study of a method of neutralizing the ammonium citrate solution, and indicate the lack of satisfaction with the present official methods. Litmus is not mentioned in the official methods, though it is doubtless regarded by a large number of analysts as being a more satisfactory indicator for determining the neutrality of this solution than corallin. Most analysts also will doubtless agree that corallin has proven somewhat unsatisfactory.

The lack of uniformity in the citrate solution in the first place must necessarily result in more or less confusion, especially in the examination of non-acidulated products, and while the certain recognition of the strict neutrality of the citrate solution has stimulated a great deal of study and experiment, there is yet evidence of a lack of general confidence in the methods which have been proposed. We must usually rely upon some kind of an indicator for the determination of the presence of appreciable amounts of hydrogen or hydroxyl ions, and no device yet proposed of using corallin for this purpose in preparing ammonium citrate has met with general satisfaction. This is evidently true also of cochineal in the Houston method, involving the previous removal of at least the greater portion of the citrate ions by alcoholic calcium chlorid.

Determination of neutrality by analysis.

The referee last year, Doctor McCandless, proposed to set the solution by determining its ratio of citric acid to ammonia, this ratio for the tri-ammonium salt being computed by him as 3.765 to 1. Acting doubtless on this method of approaching the matter, a committee of the division of fertilizer chemists of the American Chemical Society, after the analysis of a carefully prepared solution, recommended that a standard citrate solution be made to contain in one liter at 20° C. 186.4 grams of anhydrous citric acid and 43.7 grams of anhydrous ammonia, a ratio of 4.25 to 1, as against 3.765 to 1 for the tri-ammonium salt and 3.809 to 1 for the three solutions examined and found neutral by the referee in 1908. The three solutions found neutral and analyzed by the referee last year contained 168.76 grams of citric acid and 44.3 grams of ammonia.

The total average amount of ammonium citrate in the three solutions found neutral by the referee last year was 213.06 grams, and that recommended by the committee of fertilizer chemists is 229.70 grams. There is still, therefore, a lack of agreement as shown by these analyses of solutions, prepared doubtless with especial care, and we are led to inquire whether this is due to inherent errors in the method of analysis, to improperly set solutions, or to both of these causes.

It has been pointed out also that a citrate solution made according to our official methods does not contain sufficient ammonia to form in it the tri-ammonium salt only, and it appears that this is precisely what we should expect, since more or less hydrolytic dissociation undoubtedly occurs, from which it follows that a water solution of the tri-ammonium salt would give an alkaline reaction to any sensitive indicator. It therefore appears that any analytical method in a large measure leaves the question in its former condition, because we must first prepare the neutral solution before we can ascertain the precise amounts of citric acid and of ammonia that will reproduce it.

The official method requires a neutral solution, and this should demand less than the theoretical amount of ammonia for its preparation, and therefore it is difficult to see any advantage in attempting to determine by analysis whether a given solution contains certain specified amounts of citric acid and ammonia, or whether these constituents are present in a certain ratio, because of the fact already pointed out that a neutral solution must first be prepared from which

the ratios and absolute amounts of citric acid and ammonia are to be ascertained subsequently by analysis. In other words, the preparation of an accurate solution must precede the analysis.

Moreover, unavoidable errors in analyses made for this purpose produce rather large variations in the final results, since a difference of 0.10 cc of fourth-normal alkali would produce a variation of 40 cc fourth-normal ammonia per liter when 2.5 cc of the citrate solution under examination are measured out for the determination. This corresponds approximately to 0.50 cc of strong ammonia solution. In a similar way a difference of 0.20 cc of fourth-normal alkali in the titration of the citric acid would correspond to about 3.84 grams of citric acid per liter.

In addition to this, when the absolute content of citric acid and of ammonia is specified, as suggested by the committee of fertilizer chemists, the effect of variations in gravity becomes pronounced, as the determinations given below will show.

A carefully neutralized ammonium citrate solution was brought to a gravity of 1.09 by means of a hydrometer. The specific gravity was found to be 1.09077 when a pyknometer was used. The solution was diluted with successive small portions of water and gravities determined, using a pyknometer. The effect of variations in specific gravity on the content of citric acid and ammonia present in a liter is shown in the last column, the neutral solutions analyzed by Doctor McCandless last year showing an average of 213.06 grams of citric acid and ammonia.

Effect of variations in specific gravity on content of citric acid and ammonia.

Specific gravity of original solution of ammonium citrate.	Solution diluted as follows:		Specific gravity after dilution.	Weight of citric acid and ammonia in 1 liter when a solution of 1.090 sp. gr. is assumed to contain 213.06 grams.
	Volume of water added.	Volume of solution.		
	cc.	*cc.*		*Grams.*
1.09077	10	990	1.09008	213.06
1.09077	20	980	1.08858	208.76
1.09077	30	970	1.08656	206.61
1.09077	50	950	1.08490	202.30
1.09077	70	930	1.08321	198.00
1.09077	90	910	1.08161	193.70
1.09077	110	890	1.07997	189.39

These data illustrate the necessity of bringing the solution to a specific gravity of precisely 1.09 before measuring out 2.5 cc for analysis when the solution is to be made to contain definite amounts of citric acid and ammonia in a liter. Variations in gravity would produce no effect, of course, when the neutrality of the solution is determined by the ratio of citric acid to ammonia, as proposed by the referee in 1908.

Suggestions concerning a uniform method for preparing neutral ammonium citrate.

The referee believes that a slight modification of the official method of preparing a citrate solution can be made to yield satisfactory results after some practice if analysts will follow details carefully and devote especial attention to this feature of the work until they are confident that their solutions may be accurately reproduced at any time.

It can be shown that a solution of purified litmus, or, better, of azolitmin, will give evidence of the addition of 0.10 cc of seminormal ammonia to 2.5 cc, and perhaps more, of the citrate solution examined for neutrality. Up to this time litmus has been found more satisfactory than other indicators tried, and azolitmin gives excellent results when the difficulties involved are considered. The method is substantially that suggested by Lord:[a]

Select four Nessler's jars, in which equal volumes occupy precisely equal heights. Add carefully from a burette the same amount of indicator solution to each jar. To each of one pair of jars add 2.5 cc of the citrate solution already approximately neutral. To one of the remaining jars add a few drops of strong solution of citric acid, and to the other jar a few drops of strong ammonia solution. Make all of the jars up to the 50 cc mark with boiled distilled water. Place the two pairs of jars in a box with a partition through the center. Narrow slits are cut on each side of the partition, allowing the operator to compare the tints in the pairs of jars with ease and great accuracy. The jars containing the ammonium citrate to be tested are placed in one partition, and the jars showing the acid and alkali tints of the indicator are placed in the other partition. These are for comparison, and since one is alkaline and the other acid, a perfectly neutral tint will be observed when looking through both of them through the slit in the box. The slightest acidity or alkalinity is shown by a difference in tint between the two pairs of jars.

Practice will give the analyst confidence in his ability to prepare a uniform citrate solution, and as this method is extremely simple and easily carried out, and as it will certainly yield very uniform results, the referee believes that it should be given a careful trial. It has been stated already that the addition of 0.10 cc of seminormal ammonia to 2.5 cc of citrate solution may be imme diately recognized in this method of determining the point of neutrality to litmus.

UNIFORM PROCEDURE IN CARRYING OUT THE DETERMINATION OF CITRATE INSOLUBLE.

The lack of uniformity in carrying out the details of this process is well illustrated by the replies received by the referee to the inquiries already noted. The very nature of this determination, of course, requires uniformity in all essential details.

In reviewing the communications received from various analysts, we find that the digestions were carried out usually in Erlenmeyer flasks, varying in capacity from 200 cc to 600 cc, though Kjeldahl and other kinds of flasks were employed.

The flasks were immersed in the bath to various depths, the height of water varying from the level of the citrate solution in the flask to some distance above it. One analyst allowed his flask to float, and one used a submerged Kjeldahl.

The temperature of the bath water varied from 65° to 70° C., though one chemist stated that the bath was maintained at a temperature of 75° C., but this is probably an error.

Fourteen analysts filtered by suction after digestion while seven did not. Various methods of carrying out the filtrations are noted.

The filtrates secured are generally reported as free from sediment, though the referee has observed that unless a very closely woven paper is employed in making these filtrations by suction, many kinds of acid phosphates especially will be found to give cloudy filtrates, the finer particles passing through the filter.

Somewhat more striking variations are observed in washing this citrate insoluble residue, the total volume of the filtrate varying between 200 cc and 1,000 cc.

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[a] J. Amer. Chem. Soc., 1896, **18**: 457.

It has been shown by Veitch [a] that the amount of water used in washing this residue from practically all kinds of fertilizer materials is by no means unimportant. It was pointed out by him that when the total filtrate measuring 200 cc was rejected, the next 100 cc of wash water removed an average of 0.129 per cent; the next, 0.05 per cent; the next, 0.04 per cent; and the last, 0.03 per cent of phosphoric acid.

When the volume of filtrate and washings together amounted to 250 cc, the next 100 cc of wash water removed an average of 0.05 per cent, from all of which it is apparent that water at 65° C. will continue to remove phosphoric acid from these residues as long as it is applied.

Six analysts write that they employed only 100 cc of wash water, and this amount is evidently insufficient as these filtrations are ordinarily carried out, though of course when rapid and efficient filtration is secured and the wash water is applied in small portions a thorough washing may be secured by a correspondingly smaller amount of water.

Cooperative Work.

Since the use of a uniform citrate solution and uniformity in manner and temperature of digestion, washing, and quick and efficient filtration are essentials for comparable results, those who had volunteered to take part in this work were asked to make comparative determinations on several kinds of materials under definite conditions outlined as follows:

INSTRUCTIONS FOR COOPERATIVE WORK.

Description of samples.

Sample No. 1.—Acid phosphate prepared by thoroughly mixing about 60 samples of acid phosphate manufactured by various fertilizer manufacturers.

Sample No. 2.—A mixture of sample No. 1 and tankage.

Sample No. 3.—A mixture consisting of acid phosphate (sample No. 1), dried blood, tankage, and bone meal.

Sample No. 4.—Prepared by thoroughly mixing a large number of complete fertilizers in which cottonseed meal was used as a source of nitrogen.

Determination of citrate-insoluble phosphoric acid.

Preparation of neutral ammonium citrate.—Neutralize roughly a convenient quantity of commercial citric acid with diluted ammonium hydroxid (Bul. 107, Revised, p. 1, method (a)).

Add dilute ammonia or citric acid solution until the ammonium citrate appears to be practically neutral to a solution of litmus or azolitmin. Complete the neutralization as follows: [b]

Add pure litmus solution to about 200 cc of neutral distilled water until it is colored distinctly but not deeply. Take half of this and dilute further with its own volume of water. Now take three clear cc Nessler tubes, fill two of them with the diluted liquid, and the third to the same depth with the stronger solution. To one of the first two add a drop of dilute sulphuric acid, to the other a drop of ammonia. Set these tubes one in front of the other, so that the light passes through both, thus giving a strictly neutral purple color; a little care will enable one to see them almost like one tube against a sheet of white paper in a ground glass. It makes no difference which tube is in front. Now to the

[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. 49, p. 73.

[b] Lord, J. Amer. Chem. Soc., 1896, **18**: 457.

liquid in the third tube containing the stronger solution (which is obviously equal in color depth to the double thickness of the first two tubes) add 5 cc of the citrate solution to be tested, and compare the color produced with the color shown by the doubled tubes. The slightest acidity or alkalinity of the citrate is at once shown by difference of tint; the test is very sensitive.

After neutralizing the solution as above described, bring to a gravity of 1.090.

Digestion.—Place 100 cc of citrate solution, prepared as just described, in a 250 cc Erlenmeyer flask, and bring to a temperature of 65° C. Immerse the flask until the water in the bath stands at least 1½ inches above the ammonium citrate in the flask. Add the residue from the water soluble determination, and proceed with the digestion as described in the official method.

Filtration and washing.—Filter samples Nos. 2, 3, and 4 by gentle suction through a No. 597 12.5 cm S. & S. paper resting on a platinum cone.

Sample No. 1 is an acid phosphate, and should be filtered as follows: Place a 9 cm No. 590 S. & S. paper over the platinum cone and on top of this use the No. 597 paper mentioned above. With accurately fitted papers and gentle suction this method will give a clear filtrate for this acid phosphate.

If filtrates secured are not clear, please repeat the determination, using a No. 590 ashless filter under the No. 597 papers in each case.

Filter and wash with water at 65° C. until the total volume of the filtrate measures 350 cc. These filtrates must be clear. *Preserve them carefully for further treatment, as described later.*

Solution of the citrate insoluble residue.—Digest this residue in 30 cc concentrated sulphuric acid as directed under (a_3) in the official method, and determine the phosphoric acid by the gravimetric process.

ANALYTICAL DATA.

Comparative results on citrate-insoluble phosphoric acid.

Analysts.	Sample No. 1.	Sample No. 2.	Sample No. 3.	Sample No. 4.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
H. S. Chilton, Agricultural College, Mississippi	1.07	1.51	3.52	0.96
	1.10	1.56	3.43	.96
	1.07	1.51	3.39	.95
	.....	1.57	3.83	.....
	1.01	1.48	3.75	.97
	.....	.....	3.75	.....
W. Stark, Agricultural College, Mississippi	1.09	1.56	3.68	1.04
	1.23	1.62	3.38	1.01
	1.18	1.73	3.35	1.00
	1.20	1.62	3.38	1.01
	.....	.....	.....	.97
I. D. Sessums, Agricultural College, Mississippi	.....	.....	3.86	.....
	.83	1.45	3.83	.91
	.....	.....	3.61	.....
	.83	1.41	3.71	.91
	.....	.....	3.99	.....
	.....	.....	4.00	.....
	.....	.....	3.74	.....
	.....	.....	3.77	.....
	.....	.....	3.68	.....
	.....	.....	3.68	.....
L. S. Walker, Amherst, Mass.	[a].51	[a].84	[a]3.10	[a].36
	[a].46	[a].87	[a]3.12	[a].31
T. W. Holmes, New Orleans, La.	1.31	1.42	3.92	1.05
	1.35	1.45	3.95	1.10
	1.25	1.40	3.86	1.12
	1.28	1.38	3.88	1.08
F. P. Veitch, Washington, D. C.	.92	1.53	3.54	.89
	.92	1.47	3.57	.92
R. B. Deemer, College Park, Md.	[a].65	[a]1.12	[a]3.22	.73
	.85	[a]1.17	3.35	.73
	[a].78	1.30	3.35	.70

[a] Excluded from average.

Comparative results on citrate-insoluble phosphoric acid—Continued.

Analysts.	Sample No. 1.	Sample No. 2.	Sample No. 3.	Sample No. 4.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Thos. C. Pinkerton, Philadelphia, Pa	0.87	[a] 1.21	3.50	0.74
	.84	1.24	3.52	.72
F. B. Carpenter, Richmond, Va	.97	1.36	3.68	.79
	1.02	1.30	3.68	.80
P. F. Trowbridge, Columbia, Mo	1.12	1.28	3.47	.92
	1.02	1.25	[a] 3.01	[a] .54
				[a] .54
	.92	[a] 1.15	[a] 3.27	.98
				[a] .61
E. W. Magruder, Richmond, Va	.96	1.42	3.72	[a] 1.40
	.90	1.64	3.76	[a] 1.28
	.90	.....	3.70	[a] 1.30
P. Rudnick, Chicago, Ill	.80	1.37	3.88	.90
	.80	1.39	3.90	.91
				.92
Averages	1.02	1.46	3.66	.92

[a] Excluded from average.

DISCUSSION OF RESULTS.

The comparative determinations presented in the foregoing table leave much to be desired. The variations shown add emphasis to the necessity of an accurate control of uniform conditions in making these determinations. When solutions of the insoluble residue are once prepared, there should be no difficulty in the accurate estimation of phosphoric acid in them, and these differences must be due in large measure to variations in the neutrality of the citrate solution, time required for filtration, washing, etc.

The referee believes that a uniform citrate solution can be prepared by the method suggested after some practice by analysts. Its advantages are certainly worthy of careful trial. The lack of uniformity of procedure in carrying out digestions, filtrations, washings, etc., should be corrected, and while it is not desirable to specify details too minutely in methods, the lack of a reasonably uniform procedure must necessarily result in variations between analysts, which are not allowable, and certainly close attention to uniform details in this determination is necessary.

THE DIRECT DETERMINATION OF CITRATE-SOLUBLE PHOSPHORIC ACID.

That the estimation of phosphoric acid by the ordinary molybdate process can be carried out in the presence of citric acid is well known, and the direct determination of the citrate-soluble phosphoric acid in the filtrate, especially after making the digestion, was undertaken by several collaborators whose results, although encouraging, are not included in this report.

Good results were secured by Veitch [a] in the direct determination of citrate-soluble phosphoric acid, and it is believed that this matter is well worthy of further careful investigation.

It is scarcely necessary to point out the possibility of variations in the results by this method, due to the presence in the filtrates of phosphorus not in combination as orthophosphoric acid.

The results given in the following table show that in the trials made citric acid did not interfere with the quantitative precipitation of phosphoric acid, but it should be pointed out that it is very necessary to add a larger quantity of molybdate solution when citric acid is present, the excess depending upon the amount of citric acid.

[a] J. Amer. Chem. Soc., 1899, **21**: 1090.

Determinations of total phosphoric acid by the official gravimetric method with and without the addition of ammonium citrate solution.

Sample No. 1.		Sample No. 2.		Sample No. 3.		Sample No. 4.	
Official method.	Modified method.[a]	Official method.	Modified method.[a]	Official method.	Modified method.[a]	Official method.	Modified method.[a]
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
15.42	15.48	13.96	14.02	14.89	14.98	11.58	11.60
15.39	15.40	13.91	13.94	14.88	14.98	11.54	11.58
15.35	15.48	13.94	13.96	14.81	15.02	11.60	11.64
15.35	15.35	14.02	14.02	14.78	14.94	11.49	11.71
15.33	15.38	13.93	14.11	14.78	14.87	11.56	11.62
15.35	15.45	13.98	13.94	14.80	14.88	11.60	11.65
Av. 15.36	15.42	13.95	13.99	14.82	14.94	11.56	11.63

[a] Thirty cubic centimeters of official ammonium citrate added before addition of molybdic solution.

The foregoing determinations were made by H. S. Chilton and W. Stark, and indicate that as much as 30 cc of official ammonium citrate introduces no source of error in the estimation of phosphoric acid by the molybdate process when the molybdate solution is added in *excess* of the amount required when no citric acid is present. One hundred cubic centimeters were used in each of these determinations, the solution being previously heated to 80° C. before addition of the molybdic solution at same temperature.

Recommendations of Referee.

It is recommended that—

(1) The association appoint a special committee to study the availability of basic slags under American conditions. This committee to secure if possible the cooperation of the experiment stations in instituting field trials to be continued at least five years. This committee to make annual reports to the association of the progress of the work undertaken, and to make a complete report after five years, with suggestions concerning a laboratory process for estimating the availability of the phosphoric acid in basic slags.

That for the present the Wagner method of estimating availability of phosphoric acid in slags be made optional.

(2) The following method of preparing a neutral ammonium citrate solution be made optional:

Neutralize (as nearly as possible) a convenient quantity of citric-acid solution with strong ammonium hydroxid, testing with a solution of purified litmus or of azolitmin. Allow to stand over night, and dilute until the gravity is approximately 1.09, and complete the neutralization as follows:

Select two pairs of Nessler's jars in which equal volumes occupy equal heights. Add from a burette precisely the same amount of azolitmin or of purified litmus solution to each of the four jars. To each of one pair of jars add 2.5 cc of the citrate solution already approximately neutral, and bring to the 50 cc mark with boiled distilled water. These two jars are to be placed one in front of the other in observing the tint produced by the indicator.

To one of the remaining pair of jars add one or two drops of a strong solution of citric acid, and to the other one or two drops of strong ammonium hydroxid solution; bring to the 50 cc mark with boiled distilled water. Place these two jars one in front of the other for observing the *neutral* tint of the indicator, which is to be duplicated by the pair of jars containing only the indicator and ammonium citrate solution.

Add citric-acid solution or ammonium hydroxid to the approximately neutralized citrate solution until the tint produced by the indicator in the pair of jars containing it matches the neutral tint observed when looking through the pair of jars prepared for this comparison. Bring to a specific gravity of 1.09, using a Westphal balance or a pyknometer.

(3) The digestion be carried out in flasks of 200 cc capacity, preferably Erlenmeyer's, the flasks to be immersed until the water in the bath is at least 1 inch above the level of the solution in the flask. The heating of the bath is to be carefully regulated that the citrate solution may be maintained at 65° C.

(4) The citrate-insoluble residue be separated by filtration through a paper resting upon a cone, suction being employed to secure rapid action. The filtrates must be free of suspended matter.

(5) The citrate-insoluble residue be washed until the total volume of filtrate and washings is approximately 300 cc.

REPORT ON NITROGEN.

By C. H. Jones, *Referee*, and J. W. Kellogg, *Associate Referee.*

The work on nitrogen for the year 1909 was conducted in accordance with the following recommendations passed in 1908:

(3) Bulletin 107, Rev., page 8, line 4, after the word "time," insert: "Allow the flask to stand without heat for not less than six hours, or for a shorter time, with shaking at regular intervals."

(4) Bulletin 107, Rev., page 8, under "(3) Determination," line 5, after the word "and," insert the same sentence as in recommendation 3. The sentence then reads "Add 5 grams of thiosulphate and allow the flash to stand without heat for not less than six hours, or for a shorter time, shaking at regular intervals; then heat the solution for five minutes," etc.

Two samples were prepared for cooperative work, the composition of which was as follows:

Composition of samples for cooperative work.

Constituents.	Sample No. 1.	Sample No. 2.
	Grams.	*Grams.*
Nitrate of soda	200	400
Cottonseed meal	400	400
Acid phosphate	1,200	900
Muriate of potash	200	400

The crude materials were finely ground previous to mixing, and special care was used in mixing and subsampling to insure uniformity. Four samples from each lot, selected at random, gave the following nitrogen results:

No. 1, (a) 2.83 per cent, (b) 2.86 per cent, (c) 2.90 per cent, and (d) 2.83 per cent.

No. 2, (a) 4.23 per cent, (b) 4.16 per cent, (c) 4.26 per cent, and (d) 4.12 per cent.

Based on the nitrogen content of the crude stock used, namely, nitrate of soda, 15.44 per cent; cottonseed meal, 6.18 per cent; and acid phosphate, 0.06 per cent, the calculated nitrogen percentage in the samples is 2.82 per cent for No. 1 and 4.14 per cent for No. 2. The samples contain, respectively, 1.54 and 2.94 per cent of nitrogen derived from nitrate of soda.

Twenty-six sets of samples were sent out to analysts who had signified a desire to cooperate in the work, together with the following instructions:

Instructions for Nitrogen Work, 1909.

Determine total nitrogen in samples 1 and 2 as per methods stated below:

A. Place from 0.7 to 3.5 grams of the substance to be analyzed in a Kjeldahl digestion flask and add 30 cc of sulphuric acid containing 1 gram of salicylic

acid; shake until thoroughly mixed, then add 5 grams of crystallized sodium thiosulphate. Place the flask on the stand for holding the digestion flask and heat over a low flame until all danger from frothing has passed. Continue as directed on page 8, line 6, Bulletin 107, Revised.

B. Place from 0.7 to 3.5 grams of the substance to be analyzed in a Kjeldahl digestion flask; add 30 cc of sulphuric acid containing 2 grams of salicylic acid, then add gradually 2 grams of zinc dust, shaking the contents of the flask at the same time. Finally, place the flask on the stand for holding the digestion flask and heat over a low flame until all danger from frothing has passed. Continue the determination as directed on page 8, line 6, Bulletin 107, Revised.

C. Place from 0.7 to 3.5 grams of the substance to be analyzed in a Kjeldahl digestion flask; add 30 cc sulphuric acid containing 1 gram of salicylic acid; shake until thoroughly mixed, and allow to stand from five to ten minutes, with frequent shaking. Add 5 grams of sodium thiosulphate and heat the solution for five minutes; cool; add 10 grams of potassium sulphate, and continue as directed in Bulletin 107, Revised, page 8, under "(3) Determination."

D. The same as under A except that after the addition of sodium thiosulphate allow the flask to stand without heat for not less than six hours, with shaking at regular intervals.

E. Same as under B except that after the addition of the zinc dust allow the flask to stand without heat for not less than six hours, shaking at regular intervals.

F. Same as under C except that after the addition of the sodium thiosulphate allow the flask to stand without heat for not less than six hours, shaking at regular intervals.

Make duplicate determinations in each case and report individual results.

Results were received from nineteen analysts, representing sixteen laboratories, and are stated in the following table:

Cooperative work on determination of total nitrogen by six different methods.

Analyst.	Method A.		Method B.		Method C.		Method D.		Method E.		Method F.	
	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
J. E. Breckenridge, and B. W. Bangs, Carteret, N. J	2.72	4.20	2.72	4.04	2.88	4.12	2.96	4.08	2.86	4.08	2.76	4.16
	2.72	4.20	2.72	4.00	2.84	4.04	2.88	.....	2.84	4.14	2.64	4.04
V. J. Carberry, New Brunswick, N. J	2.70	3.87	2.74	4.19	2.72	4.06	2.79	3.98	2.77	4.19	2.80	4.03
A. M. Henry, Tallahassee, Fla	2.74	3.80	2.72	4.00	2.73	4.01	2.88	3.96	2.85	4.27	2.81	4.08
	2.79	3.88	2.74	4.03	2.82	3.96	2.89	3.96	2.86	4.17	2.85	4.10
J. A. Hummel, St. Paul, Minn	2.71	4.02	2.73	4.12	2.75	3.91	2.74	4.00	2.86	4.10	2.80	4.00
	2.68	4.05	2.79	4.08	2.72	3.95	2.74	3.99	2.71	4.10	2.73	4.01
T. C. Trescot, Washington, D. C	2.72	3.99	.....	.....	2.78	4.04	2.81	4.13	.....	.....	2.86	4.25
	2.67	4.04	.....	.....	2.72	4.04	2.84	4.13	.....	.....	2.89	4.18
W. D. Cook, Richmond, Va	2.72	4.07	.....	.....	2.78	4.15	2.84	4.13	.....	.....	2.86	4.15
	2.74	4.06	2.69	3.90	2.90	4.19	2.90	4.16	2.77	4.15	3.03	4.25
	2.73	4.04	2.68	3.88	2.90	4.18	2.87	4.14	2.68	4.02	2.93	4.18
	.....	.....	.....	.....	.....	.....	.....	.....	2.68	.....	2.88	.....
T. C. Pinkerton, Philadelphia, Pa	2.96	4.20	2.91	4.12	2.76	3.98	2.89	4.24	2.95	4.28	2.90	4.15
	2.96	4.12	2.88	4.23	2.78	4.03	2.91	4.24	2.96	4.31	3.01	4.12
	2.90	4.00	2.85	4.18	2.84	3.95	2.86	4.28	2.90	4.31	2.90	4.20
	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.96	.....
W. A. Branon, Madison, Wis	2.85	3.96	2.79	4.13	2.81	3.98	2.85	4.11	2.88	4.05	2.73	4.14
	2.98	3.88	2.79	3.94	2.82	4.00	2.71	4.25	2.83	4.20	2.81	4.10
	2.83	4.20	2.76	4.01	2.83	4.12	2.92	4.11	2.76	4.21	2.80	4.15
	2.88	4.28	.....	4.15	.....	3.97	2.77	4.25	2.87	4.21	.....	.....
	2.81	.....	.....	4.00	.....	.....	.....	.....	.....	.....	.....	.....
P. A. Benedict, Burlington, Vt	2.79	4.09	2.72	4.19	2.72	4.05	2.76	4.12	2.86	4.23	2.79	4.05
	2.79	4.12	2.72	4.19	2.72	4.16	2.83	4.12	2.79	4.12	2.79	4.12
	.....	.....	.....	4.19	.....	4.09	.....	.....	.....	4.16	.....	.....
	.....	.....	.....	4.00	.....	4.09	.....	.....	.....	.....	.....	.....
W. B. Derby, Burlington, Vt	2.83	4.23	2.72	4.12	2.93	4.26	2.72	4.19	2.93	4.09	2.93	4.30
	2.79	4.05	2.72	4.23	2.86	4.30	2.72	4.23	2.97	4.23	2.86	4.16
C. H. Jones, Burlington, Vt	.....	4.12	.....	.....	.....	4.09	2.83	.....	.....	.....	2.83	.....
E. P. Verner, Charleston, S. C	2.79	4.19	2.79	4.19	2.86	4.19	2.83	4.23	2.79	4.19	2.79	4.23
	2.77	3.97	2.90	4.15	2.90	4.15	2.92	4.27	2.80	4.20	2.90	4.15
	2.75	4.05	2.85	4.15	2.85	4.15	2.85	4.25	2.85	4.25	2.95	4.20

Cooperative work on determination of total nitrogen by six different methods—Continued.

Analyst.	Method A.		Method B.		Method C.		Method D.		Method E.		Method F.	
	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.	No. 1.	No. 2.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
W. D. Richardson, Chicago, Ill	2.78	4.14	2.85	4.10	2.82	4.14	2.96	4.29	2.98	4.25	2.90	4.32
J. C. Reed, Amherst, Mass	2.62	3.61	2.65	3.85	2.70	3.84	2.75	3.93	2.79	3.94	2.92	3.97
	2.59	3.62	2.65	3.84	2.70	3.85	2.74	3.92	2.81	3.93	2.73	3.96
	2.58	3.62	2.63	3.85	2.69	3.86	2.74	3.92	2.80	3.94	2.73	3.95
F. B. Carpenter, Richmond, Va	2.78	4.07	2.66	3.95	2.83	4.17	.....	.....	.....	.....	.....	.....
	2.75	4.19	2.66	3.92	2.86	4.19	.....	.....	.....	.....	.....	.....
	2.81	4.20	2.78	4.01	2.82	4.23	.....	.....	.....	.....	.....	.....
A. A. Jones, Columbia, Mo	2.80	4.05	2.72	3.99	2.71	4.08	2.76	3.91	2.64	3.90	2.76	4.14
	2.76	3.95	2.59	3.97	2.70	4.10	2.71	3.99	2.70	3.98	2.81	4.01
	2.70	3.96	2.70	4.03	2.65	3.92	2.75	3.95	2.67	3.94	2.73	4.20
Grover J. Secord, Lansing, Mich	2.79	4.21	2.90	4.07	2.72	4.11	2.76	3.94	2.88	4.18	2.62	3.85
	2.80	4.26	2.86	4.16	2.68	4.07	2.77	3.95	2.85	4.22	2.58	3.80
J. W. Kellogg, Harrisburg, Pa	2.73	3.95	.....	3.87	2.84	4.04	2.79	3.98	2.67	3.95	2.85	4.29
	2.08	3.95	.....	3.83	2.84	4.01	2.78	3.95	2.68	3.97	.....	4.20
	2.65	3.91	.....	.....	2.79	4.01	.....	.....	.....	.....	.....	.....
Paul Rudnick, Chicago, Ill	2.77	4.17	2.87	4.20	2.84	4.17	2.80	4.13	2.87	4.17	2.87	4.13
	2.63	4.06	2.80	4.03	2.63	4.10	2.77	4.10	2.84	4.17	2.66	4.03
	2.80	4.10	2.87	4.06	2.80	4.06	2.80	4.03	2.87	4.06	2.73	4.03
Average	2.76	4.04	2.76	4.04	2.79	4.07	2.82	4.09	2.82	4.13	2.82	4.11

Comments by Analysts.

J. C. Reed stated that his results were obtained by digestion over a low flame for about one and one-half hours and then with full flame until solution became colorless. In most cases it was found necessary to add 10 cc of sulphuric acid to prevent the complete drying of the contents of the flasks.

In addition to the results reported in the preceding table T. C. Trescot furnished data showing the effect of standing, before heating, for one, two, and four hours and overnight by procedure C as follows:

Effect of standing before heating on results by Method C (Trescot).

Sample.	1 hour.	2 hours.	4 hours.	Overnight.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	2.80	2.86	2.78	2.84
	2.80	2.78	2.81	2.81
	2.75	2.81	2.85	2.84
2	4.04	4.15	.....	4.15
	4.07	4.18	4.15	4.18
	4.15	4.17	4.18	4.18

Paul Rudnick reports the following nitrogen results obtained by using the combined Kjeldahl-Gunning method with thiosulphate as the reducing agent: Sample No. 1: 2.92, 2.95, 3.03, and 3.03 per cent. Sample No. 2: 4.22, 4.26, 4.28, and 4.28 per cent.

Discussion of Results.

The results are not very satisfactory, variations among the different analysts being exceptionally wide, particularly with sample No. 2. The following table shows the extremes obtained by each modification:

Extremes obtained in the determination of total nitrogen by six methods.

Method.	Sample No. 1.			Sample No. 2.		
	Minimum.	Maximum.	Difference.	Minimum.	Maximum.	Difference.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
A	2.58	2.98	0.40	3.61	4.28	0.67
B	2.63	2.91	.28	3.83	4.23	.40
C	2.63	2.93	.30	3.84	4.30	.46
D	2.71	2.96	.25	3.91	4.29	.38
E	2.64	2.98	.34	3.90	4.31	.41
F	2.58	3.01	.43	3.80	4.32	.52

The greatest difference between the maximum and minimum, considering all the modifications of methods, was 0.43 per cent on sample No. 1 and 0.71 per cent on sample No. 2. The extreme variations noted can not be wholly due to differences in the samples. Seven different sets analyzed in this laboratory by four analysts showed a difference of but 0.25 per cent.

The differences between results by the several modifications of the Kjeldahl and Gunning methods by individual analysts are not so marked. The general tendency seems to be toward higher figures when methods D, E, and F are employed, though some chemists report practically similar results by all procedures.

During the past year considerable attention has been given in the Vermont station laboratory to the effect of allowing the sample to stand overnight before heating, after the addition of zinc. Sixty-one samples of nitrated commercial fertilizers were used in the comparison. Their total nitrogen content varied from 1.12 to 8.95 per cent and the nitrate nitrogen present from 0.20 to 7.74 per cent, with an average of 1.06 per cent. Methods B and E as outlined were used, save that in method E the material and reagents, after the addition of zinc, stood overnight. The average nitrogen content of the 61 samples by method B was 2.976 per cent; and by method E, 2.982 per cent. In no case was a variation of over 0.10 per cent observed.

There seems to be a tendency among certain analysts to shorten the time required for the digestion process, according to the color of the solution and the rapidity with which it clears. It is the opinion of the referee that digestion for two and one-half to three hours is necessary in all cases where the modified Kjeldahl or Gunning method is employed. It is also to be noted that it is essential that the flasks be thoroughly dry before using.

Recommendations.

It is recommended that the following changes in the methods be made:

(1) Bulletin 107, Revised, page 8, line 4, after the word "time," insert: "Allow the flask to stand without heat for not less than six hours,[a] or for a shorter time, with shaking at regular intervals."

(2) Bulletin 107, Revised, page 8, under "(3) Determination," line 5, after the word "and," insert: "allow the flask to stand without heat for not less than six hours,[a] or for a shorter time, with shaking at regular intervals."

[a] When the nitrate nitrogen present exceeds 1.50 per cent the six-hour interval is recommended.

DETERMINATION OF AMMONIA BY THE OFFICIAL MAGNESIUM OXID METHOD.

By T. C. Trescot.

In the determination of ammonia by the official magnesium oxid method it is directed that 100 cc of the liquid be distilled into standard acid, and that the residual acid be titrated as in the Kjeldahl method. Numerous determinations by this method in our laboratory have shown that for many substances the results are very unsatisfactory and can hardly be reported as free ammonia. The titration of the residual acid, when carried on as in the Kjeldahl method, using cochineal as an indicator, gives an uncertain end point, due to magnesium carbonate which is always present in the magnesium oxid. This difficulty can be entirely obviated, however, either by igniting the magnesium oxid immediately before using, or, better, as is our custom, by substituting congo red for the cochineal.

The real difficulty found in using the method arises from the fact that when the distillation as directed in the official method has been completed, if more water is added to the distillation flask and the operation continued, using a fresh portion of acid, it will be found that a part of the acid has been neutralized. This is true for many substances, as cottonseed meal, meat, eggs, and wheat; and ammonia continues to be liberated for several such distillations. It is evident that all the ammonia existing as free ammonia or ammonium compounds would be liberated by the first distillation and that other nitrogen compounds are therefore broken up with the formation of ammonia or other similarly reacting substances.

It appears, therefore, that the result of the magnesium oxid distillation should not be reported as free ammonia, but should be expressed simply as ammonia obtained by distillation of the sample with magnesium oxid. In fertilizer analyses these differences have little, if any, significance, but with other materials, notably in physiological work, they are of considerable importance.

The following table shows the percentages of nitrogen as ammonia obtained from successive distillations of a number of substances:

Nitrogen as free ammonia, determined by successive distillations by the magnesium oxid method.

Material.	Time of distillation.	First distillation.	Second distillation.	Third distillation.	Fourth distillation.	Fifth distillation.	Sixth distillation.	Total.
Ammonium sulphate	*Minutes.*	*Per cent.* 21.100	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.* 21.100
Tankage(8 years old)	40	.351	0.000					.351
Cottonseed meal	40	.042	.030					.072
Beef peptonoids	40	.056	.042	0.042	0.042			.182
Extract of beef	40	.197	.000	.000				.197
Fresh meat	20	.027	.009	.009	.004	0.000		.049
Fresh duck	25	.027	.009	.009	.005	.005		.055
Fresh quail	25	.027	.019	.008	.008			.062
Fresh chicken	30	.029	.018	.012				.059
Fresh fish	30	.013	.006	.003	.003			.025
Fresh eggs (white)	35	.013	.008	.003				.024
Fresh eggs (yolk)	35	.022	.013	.009				.044
Flour	45	.000						.000
Oats	35	.030	.014	.014	.030	.030		.118
Dried blood	40	.084	.042	.042	.042	.030	.030	.270
Corn	45	.000						.000
Ground wheat	40	.030	.042	.030	.042	.030	.030	.204

REPORT ON POTASH.

By B. B. Ross, *Referee.*

The scope of the work for 1909 has been limited to comparative tests of the official method and the cobalt-nitrite volumetric method as modified by Drushel. Twenty-six laboratories requested samples for cooperative work, but reports have been received from only eight analysts. A number of chemists who were desirous of participating in the work stated that fertilizer analyses being still in progress in their respective laboratories it would be impossible to complete the required work in time for the Denver meeting.

The use of sodium cobalti-nitrite in the quantitative estimation of potash was originally suggested by Adie and Wood [a] who gave results of both gravimetric and volumetric determinations with this reagent. It was claimed in their original paper that highly satisfactory results could be secured by the employment of this method in the analysis of various commercial potash salts, as well as certain peaty soils.

In the method of Adie and Wood, the potash was precipitated from solutions of only moderate concentration in the presence of acetic acid. A number of analysts who tried the method complained that the results secured varied with the concentration of the solution, and finally Drushel [b] proposed to evaporate the solution on the water bath to a pasty consistency after addition of the cobalti-nitrite reagent.

Adie and Wood decomposed the colbalti-nitrite precipitate by boiling with sodium hydroxid solution, the precipitated cobalt hydroxid being filtered off and washed, while the nitrite radical in the filtrate was titrated with decinormal permanganate. On the other hand, Drushel oxidizes the precipitate directly with nearly boiling permanganate solution, sulphuric acid being added toward the last to hasten the oxidation. An excess of oxalic acid is next added and the solution is then titrated to color with permanganate.

Two samples were sent to the chemists taking part in the cooperative work, one being a potassium salt of tested purity, while the other sample was a complete mixed fertilizer, accompanied by the following instructions:

Outline of Association Potash Work.

Sample No. 1.—Potassium chlorid of tested purity.

Sample No. 2.—A complete mixed fertilizer, containing a considerable proportion of organic materials.

Potash in these samples is to be determined by the official method and also by the proposed volumetric cobalti-nitrite method.

PROPOSED VOLUMETRIC METHOD, DRUSHEL'S MODIFICATION.

Reagents.

Sodium cobalti-nitrite solution (Adie and Wood).—Two hundred and twenty grams of sodium nitrite are dissolved in 400 cc of water; 113 grams of cobalt acetate are dissolved in 300 cc of water and 100 cc of glacial acetic acid added. The two solutions are mixed and gently warmed, NO_2 is evolved, and the solution becomes dark colored. The NO_2 is best evacuated from the bottle by a water pump and the liquid is left overnight, during which a yellow precipitate settles. The solution is then filtered and diluted with water to a liter.

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[a] J. Chem. Soc., 1900, 77: 1076.

[b] Amer. J. Sci., 1907, **24**: 433; Chem. News, 1908, **97**: 124.

Standard solutions.—Tenth-normal potassium permanganate solution and tenth-normal oxalic acid containing 50 cc of concentrated sulphuric acid per liter.

Method of procedure.

The solution of a potassium salt, containing not more than 0.2 gram of potassium oxid and free from ammonium salt, is treated with a rather large excess of sodium cobalti-nitrite solution, acidified with acetic acid, and evaporated to a pasty condition over the steam bath. It is then cooled and treated with from 50 to 100 cc of cold water, and stirred until the excess of sodium cobalti-nitrite is dissolved. It is allowed to settle and is decanted through a perforated crucible fitted with an asbestos felt. The precipitate is washed two or three times by decantation, after which it is transferred to the crucible and thoroughly washed with cold water. In the meantime a measured excess of standard potassium permanganate is diluted to ten times its volume and heated nearly to boiling. Into this the precipitate and felt are transferred and stirred, after which the crucible is also put into the solution, since particles of the precipitate stick persistently to its sides. After the oxidation has proceeded five or six minutes manganese hydroxid separates and the color of the solution darkens. At this point from 5 to 25 cc of sulphuric acid (1:2) are added, and the solution, after stirring, is allowed to stand a few minutes. Then a measured amount of standard oxalic acid, containing 50 cc of strong sulphuric acid per liter, is run in from a burette, taking care to add an excess. The temperature is maintained a little below the boiling point until the solution becomes colorless and the manganese hydroxid is completely dissolved. It is then titrated to color by permanganate in the usual manner. From the whole amount of permanganate employed, the permanganate equivalent of the oxalic acid used is subtracted and the remainder multiplied by the factor calculated for the strength of permanganate used, 0.000856 being the factor for strictly tenth-normal potassium permanganate.

In the analysis of mixed fertilizers the addition of cobalti-nitrite solution should be made after ignition with sulphuric acid and solution of the residue in water.

The referee suggests that an amount of solution corresponding to 0.1 to 0.2 gram be used for sample No. 1 and one gram for sample No. 2. Ten cc of the cobalti-nitrite reagent should be used for each determination.

O. M. Shedd, of the Kentucky experiment station,[a] suggests that the precipitate and asbestos felt after washing be returned to the dish in which precipitation was effected and treated with permanganate. He claims that a longer time than is suggested by Drushel should be employed in the oxidation of the cobalti-nitrite, owing to the interference of the asbestos felt with the process, and he further advises that the cobalti-nitrite be added slowly in effecting the precipitation of the potash. (Further details can be noted by referring to Shedd's original paper.)

The referee suggests that iron wire be used as an additional check in testing strength of permanganate solution.

Mr. E. L. Baker, associate referee, reports that the best results from the method have been secured by using a half-saturated salt solution for washing. It is therefore suggested that results be reported both by the use of water and of half-saturated salt solution for washing the precipitate. Moisture determinations should be reported in both samples.

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[a] J. Ind. Eng. Chem., May, 1909.

Following are the results reported by cooperating chemists:

Comparison of the official and the cobalti-nitrite methods for potash determinations.

Analyst.	Sample No. 1.		Sample No. 2.	
	Official method.	Cobalti-nitrite method.	Official method.	Cobalti-nitrite method.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
E. L. Baker, Geneva, N. Y	62.56	[a] 62.31	3.62	3.55
		[b] 60.07		
J. E. Breckenridge, Carteret, N. J			3.77	3.83
			3.79	3.83
C. E. Bradley, Corvallis, Oreg	63.12	63.14	3.52	3.60
	63.14	63.21	3.56	3.64
				[b] 3.65
				[b] 3.46
G. F. Lipscomb, Auburn, Ala		63.44	3.80	[c] 3.54
		63.34	3.85	[c] 3.56
			3.89	[c] 3.65
				[a] 3.63
				[a] 3.85
				[a] 3.90
				[a] 3.80
T. C. Pinkerton, Philadelphia, Pa			3.15	[a] 3.51
			3.13	[a] 3.54
				[b] 3.58
				[b] 3.58
P. Rudnick, Chicago, Ill. (analytical work done by F. Fenger, K. J. Monrad, and S. E. Lunak)	62.64	[a] 62.20	3.60	[a] 3.81
	62.35	[a] 62.80	3.65	[a] 3.81
	62.60	[b] 61.70	3.55	[b] 3.90
		[b] 61.70		[b] 3.93
F. P. Veitch, Washington, D. C	63.17		3.86	
	63.21		3.91	
	63.10		3.93	
	63.01		[d] 3.88	
	63.21		[d] 3.90	
	63.13		[d] 3.91	
O. M Shedd, Lexington, Ky	63.16		3.67	3.45
	63.14		3.64	3.49
	63.15		3.65	3.73
		60.75		3.30
		60.08		3.41
		63.03		3.47
(Used 0.1 gram aliquots for No. 1 and added acetic acid with nitrite reagent)		61.86		
		60.72		
		60.80		
		61.18		
Mean		61.21		
I. Used 0.2 gram No. 1, with 1 cc 50 per cent acetic acid		61.77		
II. Used 0.2 gram No. 1, without acetic acid		62.76		
III. Used 0.1 gram No. 1, without acetic acid, and evaporated to hard dryness		63.69		
IV. Like II, on 0.2 gram		63.31		
		64.44		
V. Like II, using 0.1 gram		62.76		
		62.02		
VI. Similar to III, on 0.1 gram		62.36		
		63.17		
G. Edgar, Lexington, Ky.:				
		63.05		
Used 0.2 gram aliquots		63.14		
		63.26		
Used 0.1 gram aliquot		63.09		

[a] Washed the cobalti-nitrite precipitate with water.
[b] Washed the cobalti-nitrite precipitate with half-saturated salt solution.
[c] In these determinations the potash solution was of a dilution of about 50 cc at the time the reagent was added, and hence the evaporation was more protracted. By evaporating to about 15 cc before adding the reagent, higher results were secured.
[d] These results were secured by washing 10 grams of material with boiling water to 480 cc.

As several of the cooperating chemists failed to report results for moisture determinations, and as all results reported agreed quite closely, the figures given in the table represent the results of determinations of potash in the samples as received by the analysts.

Comments by Analysts.

J. E. Breckenridge, Carteret, N. J., also determined potash in sample No. 2 from a solution prepared in the following manner: Wash 2 grams of sample on an 11 cm filter paper with small portions of hot water into a 200 cc flask until about 175 cc have run through. Add to washings about 5 cc of concentrated hydrochloric acid, heat to boiling, add ammonia and ammonium oxalate and proceed as in official method. Result was 3.94 per cent of potassium oxid.

C. E. Bradley, Corvallis, Oreg.—In our hands the factor 0.000586 is too high and the results given were calculated on the factor 0.000845. The permanganate used was checked against purified oxalic acid and iron wire. Blanks were allowed for reagents, and carried out exactly as in the determination. Results noted by this method were obtained by washing the precipitate with cold water. Originally I decomposed the yellow precipitate by the immediate addition of sulphuric acid (1 to 3) to the diluted permanganate. Care has to be exercised in the use of sulphuric acid to prevent its action on the permanganate and loss of oxygen. I have found, however, that if the temperature is not raised above 70° there is no loss of oxygen by this process; the oxidation can also be carried on somewhat more rapidly. I think, however, heating of the permanganate and completing the oxidation with sulphuric acid is more accurate. I prefer not to have a larger quantity of potassium oxid than 0.05 gram for a determination, as the washing of the precipitate can be carried on more advantageously under these conditions; it is also preferable to add the nitrite reagent to the concentrated cool solution of potash.

F. P. Veitch, Washington, D. C.—With reference to washing out the potash with hot water, rather than boiling with water, I would state that several years ago we tried this procedure and found that on some samples the results were from 0.2 to 0.3 per cent higher than by the official method, while with other samples the figures were practically identical. By washing out with a hot solution of ammonium sulphate and sodium chlorid, and also by boiling with solutions of several strengths, it was found that washing with a 1 per cent solution of ammonium sulphate almost invariably gave higher results than the official method.

O. M. Shedd, Lexington, Ky.—When the potassium salt was filtered in the volumetric method, there was great difficulty in obtaining a clear filtrate when working on the potassium chlorid. In fact, the precipitate ran through in every case with one exception, while with the mixed fertilizer a clearer filtrate was obtained, but the trouble here was that the gooch would clog and it took a long time to filter. This is probably due to the phosphate present. The results obtained on 0.2 gram aliquots by not adding acetic acid and evaporating until the residue is a thick paste on cooling, are more consistent, but not as uniform as they should be in accurate work. From a comparison of the last six results given in the table with those first reported, one can readily see that the error must be in one of the two places, or probably both are contributing causes. The difficulty is that the nitrite reagent being unstable, tends to decompose before the potassium salt is precipitated and, if this decomposition can be prevented, the separation of the potassium salt will, of course, be more complete.

In my first work, I dissolved 5 grams of potassium chlorid in 500 cc and there is a chance of error in aliquoting when working on the pure salt that one would not meet with in an ordinary fertilizer.

P. Rudnick, Chicago, Ill.: While the time given to this work was extremely limited, we are very favorably impressed with the cobalti-nitrite method. If the precipitation is made near the boiling temperature the precipitate is very easily handled, but the proportion of reagents and sample given in the method sent us required considerable adjustment before results comparing fairly well with the official method could be obtained.

Conclusions.

Reports were received from so few laboratories, relatively, that it is difficult to draw absolutely definite or satisfactory conclusions from the data presented. It will be noted, however, that most of the analysts report a fairly close agreement between the results secured by the two methods, and in a number of cases a very close concordance in the results will be observed. In

fact, the agreement between the official and the cobalti-nitrite method is much closer than was noted heretofore between the official and the phosphomolybdic methods.

Several chemists report rather low results on Sample 2, both by the official and the proposed volumetric method, and it is presumed that these low figures are due to a failure to get all of the potash in solution, as the results check each other quite well in most cases.

The referee is of the opinion that it may be possible to develop the cobalti-nitrite process into a check method that will prove of some utility and hence recommends that it be given a further trial during the ensuing year.

REPORT ON SOILS.

By S. D. Averitt, *Referee.*

Plan of Investigation.

The work recommended on soils by the association this year is a continuation of last year's work, and is mainly directed to testing provisional methods for total phosphorus and potassium.

Two soils were selected for this work. No. 1 is No. 2 of last year, and No. 2 is a low phosphate soil of Christian County, Ky. The agricultural as well as the chemical characteristics of these soils are known and they are well suited to the work in hand at this time.

The sodium peroxid fusion for total phosphorus and the modified Smith method for total potassium have monopolized a large share of the soil work for the past four years, and if with the results of this lengthy investigation, representing the work of 15 chemists, before it this body fails to make some final disposition of these methods, it will react unfavorably upon one of the main lines of investigation of the association.

In accordance with the recommendations the referee prepared and sent to about 20 chemists, who had signified a willingness to aid in this work, samples of the soils selected. The following circular letter was forwarded one day in advance of the samples:

Dear Sir: Your name appears upon the list of volunteers for association soil work this year, and I am sending you by mail two samples of soil numbered 1 and 2.

The recommendations for soil work are given on page 3, Circular No. 43, Bureau of Chemistry.

The fourth recommendation will be taken care of by Dr. J. G. Lipman, the associate referee, who will send samples and instructions for that work.

(a) It is desired that the modified J. L. Smith method for total potassium be tried on soil No. 2. The details of this method will be found on page 4, Circular 32, Bureau of Chemistry, or (d) page 90, Proceedings of 1907.

It is not necessary to wash free of chlorids, but wash five or six times by decantation with hot water. Then throw on filter and wash well; 200 to 250 cc of wash water is sufficient. Check these determinations by the regular J. L. Smith method. Make blank determinations, deducting the potassium found in reagents.

The magnesium nitrate and peroxid fusion methods for total phosphorus are to be compared on both soils.

(b) Instructions for the sodium peroxid fusion method will be found on page 234, Bulletin No. 107, Revised, Bureau of Chemistry, or page 145, Proceedings of 1906 (Bul. 105).

(c) Instructions for the magnesium nitrate method will be found on page 4, Circular No. 43, Bureau of Chemistry (the word *salt* in the first line should be *soil*).

(d) Make moisture determinations (official method) and report percentages of potassium and phosphorus on water-free basis.

If the references given are not at hand and can not be obtained readily, advise me at once and you will be supplied with a typewritten copy of the instructions.

The referee will be glad to have comments on these methods to present to the association.

Report results as soon as possible.

S. D. Averitt, *Referee on Soils.*
Jacob G. Lipman, *Associate Referee.*

ANALYTICAL RESULTS.

While some chemists have not been able to complete the work in time for the meeting, quite a large number have reported, and the referee is very much pleased with the results that have been sent to him and desires to thank the chemists who have aided in the work. Results obtained by only one method are not included in the general average.

Comparison of the sodium peroxid fusion and magnesium nitrate methods for total phosphorus.

[Water-free basis.]

Analyst.	Soil I.		Soil II.	
	Sodium peroxid fusion method.	Magnesium nitrate method.	Sodium peroxid fusion method.	Magnesium nitrate method.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
P. E. Brown, New Jersey	0.225	0.217	0.042	0.042
	.217	.215	.048	.042
	.228	.225	.....	.....
	.225	.222	.....	.....
Average	.224	.220	.045	.042
W. P. Kelley, Hawaii	.....	.223	.....	.044
	.....	.224	.....	.045
Average	.....	.224	.....	.044
O. M. Shedd, Kentucky	.....	.231	.....	.041
	.....	.231	.....	.041
	.....	.230	.....	.042
	.....	.231	.....	.042
Average	.....	.231	.....	.042
S. D. Averitt, Kentucky	.219	.231	.046	.044
	.225	.231	.042	.041
	.225	.227	.042	.042
Average	.223	.230	.043	.042
A. W. Hansen, Illinois[a]	.205	.210	.044	.045
W. B. Ellett, Virginia	.207	.252	.036	.044
	.258	.230	.040	.048
	.....	.227	.....	.....
Average	.232	.236	.038	.046
H. H. Hill, Virginia	.265	.205	.043	.042
	.265	[b].170	.041	.042
	[b].302	.....	.....	.044
Average	.277	.188	.042	.043

[a] Duplicates not reported.
[b] Not included in the general average.

Comparison of the sodium peroxid fusion and magnesium nitrate methods for total phosphorus—Continued.

[Water-free basis.]

Analyst.	Soil I.		Soil II.	
	Sodium peroxid fusion method.	Magnesium nitrate method.	Sodium peroxid fusion method.	Magnesium nitrate method.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
T. E. Keitt, South Carolina		0.215		0.039
		.216		.040
		.216		.043
		.217		.038
Average		.216		.040
J. W. Ames, Ohio		.201		.037
		.209		.037
		.207		
Average		.206		.037
W. F. Pate, Ohio		.207		.033
		.208		.034
Average		.208		.034
E. W. Gaither, Ohio		.210		.040
		.204		.036
Average		.207		.038
F. W. Woodman, Missouri	.213	0.234	[a].061	[a].061
	.268	.238	[a].057	[a].060
Average	.240	.236	.059	.060
L. D. Haigh, Missouri				.039
				.043
Average				.041
General average	.232	.226	.042	.043

[a] Not included in the general average.

Several chemists of this association have expressed doubt as to the accuracy of the volumetric determination of phosphorus. The writer has been perfectly satisfied as to its accuracy for a number of years, and a few figures obtained by him may be of interest to the members of the association at this time.

It was thought desirable to know whether all of the phosphorus could be recovered from a solution whose phosphorus content was known. For this purpose C. P. hydrogen disodium phosphate ($Na_2HPO_4+12\ H_2O$) was recrystallized from a saturated solution, and the crystals well dried between filter papers. It was intended to make a solution such that 50 cc should contain approximately 0.005 gram of phosphorus. The following determinations were then made on this solution:

Two 50 cc portions were evaporated, dried, and ignited to constant weight. I=0.0203 sodium pyrophosphate ($Na_4P_2O_7$)=0.00473 P. II=0.0206 sodium pyrophosphate ($Na_4P_2O_7$)=0.00480 P. Average=0.00476 P.

Mr. George Roberts, the fertilizer chemist of the Kentucky station, and a very careful analyst, determined the phosphorus gravimetrically on the 50 cc portions, with the following results: Weight of magnesium pyrophosphate ($Mg_2P_2O_7$), (a) 0.0163=0.00464 P, (b) 0.0167=0.00465 P, average=0.00465 P.

Volumetric determinations on 50 cc portions: (a) 0.00475 P, (b) 0.00475 P, (c) 0.00479 P.

The sodium phosphate was dissolved out of the crucible in the second determination with concentrated hydrochloric acid and the phosphorus determined volumetrically, giving 0.00475 gram. The average of all volumetric determinations was 0.00476 gram of phosphorus; theory, 0.00476 gram; gravimetric determination, 0.00465 gram. These figures speak for themselves and need no comment further than to say that the phosphomolybdate was precipitated in the usual way for soils, allowed to stand overnight, and washed with cold water.

Comparison of the Smith and modified Smith methods for total potassium on Soil II.

[Water-free basis.]

Analyst.	Smith method.	Modified Smith method.	Analyst.	Smith method.	Modified Smith method.
	Per cent.	*Per cent.*		*Per cent.*	*Per cent.*
P. E. Brown, New Jersey	1.646	1.688	S. D. Averitt, Kentucky.....	1.670	.....
	1.672	1.693		[d]1.702	1.673
				1.667	1.632
Average	1.659	1.690		1.718	1.648
				1.738	1.615
A. W. Hansen, New York....	[a]1.702	1.664		1.696	1.641
W. B. Ellett, Virginia.....	[a]1.566	1.591		1.670	1.586
W. W. Hill, Virginia.....	[b]1.900	1.599		[d]1.628	1.577
O. M. Shedd, Kentucky[c].....	1.639	1.643		[d]1.725	.....
	1.652	1.602		1.689	.....
	[d]1.659	[d]1.618		1.689	.....
	[d]1.675	[d]1.573		1.660	.....
	[d]1.694	[d]1.624			
	1.659	[d]1.598	Average	1.688	1.625
	1.631	1.682			
	[d]1.646	1.672	C. K. Francis, Missouri.....	1.700	[e]2.026
	[d]1.678	1.717		1.576	[e]2.009
	[d]1.752	1.653		1.678	.....
	[d]1.746	1.605			
	[d]1.730	1.592	Average	1.651	2.017
		1.560			
		1.605	General average.....	1.677	1.629
Average.....	1.684	1.625			

[a] Duplicates not reported.
[b] Not included in the general average; no duplicates reported.
[c] In the first five determinations 1 gram was used and divided for the regular Smith and the modified methods.
[d] Blast used in the fusion.
[e] Not included in the general average.

Comments by Analysts.

J. W. Ames: We have used the peroxid fusion method in our laboratory, but with varying success. I prefer the magnesium nitrate method, on account of the ease of manipulation and the uniform results obtained.

E. W. Gaither: The magnesium nitrate method of solution has the advantage of being easy to manipulate, giving concordant results, permitting the use of larger quantities of soil for analysis, and in saving a great amount of time.

T. E. Keitt: I only tried one of the methods, Method C (magnesium nitrate method for total phosphorus), and I wish to say that I am very much pleased with it.

W. P. Kelley: I determined the phosphoric acid by the magnesium nitrate fusion method only. It is extremely easy to work and is, I believe, a valuable method.

O. M. Shedd: It has been my experience that the modified method for total potassium will generally show slightly lower results, due, I think, to the fact that when the evaporation is being made the caustic lime separates out in flakes or scales, never granular, which occlude potassium and are very hard to wash out. Everything considered, I prefer the regular J. L. Smith method, for, while it may possibly take a little more time, it gives a more granular double potassium salt to filter and one that is not so liable to wash through as that obtained by the modified method.

Discussion of Results.

The attention of the association is called to the fact that the results obtained by the sodium peroxid fusion method for total phosphorus and by the modified J. L. Smith method for total potassium are in perfect agreement with the results obtained by these methods in 1907 and 1908.

The magnesium nitrate method for total phosphorus has been used by the referee for three years with the greatest success, and, in his opinion, leaves little to be desired. It is quick, easy, and accurate. Including this year, it has been before the association two years, about 20 chemists have tested it, and there has not been a single unfavorable comment upon it. Reference to the first table will show that quite a number of the chemists worked this method alone, which is as strong a comment as could be made in its favor.

The sodium peroxid fusion method will give accurate results, as is shown in the first table, and by the work of previous years, but it lacks ease and quickness of manipulation. One station has used this method extensively and with success, and if one prefers to use it he should do so and the association should give it recognition.

In view of the facts brought out by several years of cooperative work, the recommendations made below by the referee seem to be thoroughly warranted. The general average of results obtained by the two methods, as seen in the table (p. 27), are practically identical.

In the second table it will be noticed that while the general average of results is slightly lower by the modified Smith method, in many instances the results obtained are slightly higher than by the regular Smith method. It will also be seen that the difference between the highest and lowest results obtained by the same analyst by either method is as great as 0.15 per cent. This, in the opinion of the referee, is due to the difference in the heat employed in the fusion rather than to any subsequent feature of the determination. The work of the Kentucky station was done, using, for the most part, Lawrence Smith crucibles and the heat of the blast, or the highest heat of a special arrangement of the Bunsen burner surrounded by a jacket with the crucibles extending into the sides through thick asbestos pads. The table shows slightly higher results in most cases where the blast was used.

While the general average of results in the modified method is 0.05 per cent lower than in the regular Smith method, when taken in connection with the results of previous years it shows a remarkably close agreement with the average results of the regular Smith method, and, as stated last year, it is somewhat shorter.

Washing free of chlorids, as recommended in the modified method for total potassium, has been found to be entirely unnecessary, entailing a loss of both time and work, as the washings show chlorids long after careful spectroscopic tests fail to show potassium in the residue. The washing recommended by Smith in his original paper is ample.

Before leaving the subject of total potassium in soils the attention of the association is called to the Drushel modification of the cobalti-nitrite method in connection with the Smith fusion for total potassium. This method will be presented in a supplementary paper, and, from his experience with it, the referee feels that it should be tested by the association.

Recommendations.

It is recommended that—

(1) The magnesium nitrate method for total phosphorus be made official.

(2) The sodium peroxid fusion method for total phosphorus be made an optional official method.

(3) The modified Lawrence Smith method for total potassium be made official.

(4) The clauses "and transfer to a filter" and "after washing free of chlorids" in the modified J. L. Smith method for total potassium be replaced by the following sentence: After washing 4 or 5 times by decantation through a filter with hot water, throw on the filter and wash well, 250 to 300 cc of wash water being sufficient.

(5) The Drushel modification of the cobalti-nitrite method in connection with the J. L. Smith fusion be tested by the association the coming year as a method for total potassium. (See Journal of Industrial and Engineering Chemistry, May (No. 5), 1909.)

REPORT ON THE DETERMINATION OF CARBONATES IN SOILS.

By Jacob G. Lipman, *Associate Referee.*

Cooperative Work.

In accordance with the recommendations of 1908 the associate referee made a further test of the value of the Knorr method for determining carbonates in soils. Two samples, neither of them high in carbonates, yet possessing well-marked differences, were sent out to those who had volunteered for cooperative work on soil methods. Only five chemists reported results, namely, Brown of New Jersey, Ellett of Virginia, Greaves of Utah, Hansen of Illinois, and Hill of Virginia.

Determination of carbon dioxid in soils.

[Percentage of dry soil.]

Analyst.	Soil No. 1.	Soil No. 2.	Analyst.	Soil No. 1.	Soil No. 2.
P. E. Brown, New Jersey	0.070	0.027	J. E. Greaves, Utah	0.072	0.023
	.073	.028		.073	.021
	.065	.031		.075	.023
	.072	.024		.....	.020
	.068	.027		.....	.022
	.073	.....			
Average	.070	.027	Average	.073	.022
W. B. Ellett, Virginia	.085	.027	A. W. Hansen, Illinois	.092	.027
	.082	.035	H. H. Hill, Virginia	.092	.027
	.....	.028		.085	.027
	.....	.035		.085	.030
				.082	.....
Average	.083	.031		.085	.....
			Average	.086	.028
			General average	.081	.027

The results which are presented in the table indicate that the Knorr method when used in the proper way may give satisfactory results with soils containing less than 0.1 per cent of calcium carbonate or its equivalent. It will be seen that for Soil 2 the agreement among the several chemists is very good. Excluding the results secured by Greaves the differences are very slight. On the other hand, the analytical differences on Soil 1 are considerably wider. Both Brown and Greaves obtained lower figures than the other analysts, the average found by Brown being 0.070 per cent, and that by Hansen 0.092 per cent. There was thus a difference of more than 0.020 per cent on the general average of 0.081 per cent. Such differences, while not serious in themselves, are somewhat too large for exact soil analytical work. The sources of error lie, for the most part, in the too rapid bubbling of the gases through the potash bulbs,

in the accumulation of too much moisture in the soda lime tube attached to the potash bulb, and in the weighing operations.

It is suggested that the acid and soil be heated very gently at first, so that the evolved gases and vapors will pass slowly through the potash solution. Heating should continue for about fifteen minutes and aspiration for the same length of time. Only 10 grams of soil should be used for each determination, the sulphuric acid and soda lime should be renewed frequently, and the weighing should be done carefully. By observing the precautions just indicated, the determination of carbonates in soil may be made with a fair degree of accuracy. It is not unlikely, however, that the Knorr method could be modified to advantage by titrating the carbon dioxid evolved with barium hydrate and very dilute sulphuric acid, instead of making the determination by direct weighing as at present. For the time being the Knorr method could be recommended as a provisional method.

F. T. Shutt has transmitted the results secured by A. J. Charron, of the Central Experimental Farm, Ottawa, Canada. The results secured by Charron, recalculated on the percentage basis, were as follows:

Determinations of carbon dioxid in soils using different amounts of the sample (Charron).

Soil 1.		Soil 2.	
On 10 grams of soil.	On 50 grams of soil.	On 10 grams of soil.	On 50 grams of soil.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
0.080	0.065	0.036	0.021
.074	.....	.036	.022
.080	.....	.030	.020
.080	.....	.....	.....
Av. .079	.065	.034	.021

Comparing these results with those secured by the other analysts it is seen that on the 10 gram samples the agreement is satisfactory for Soil 1. For Soil 2 the results obtained by Charron are higher than those reported by the other analysts. On the other hand, the results secured on the 50 gram samples are considerably lower for both soils.

In commenting on the use of 10 grams of soil for carbon dioxid determinations Charron says: "The quantity of carbon dioxid found in this amount of soil was so small that its determination was considered almost nugatory. Further determinations were made on larger quantities (50 grams). * * * It is noteworthy that the amount of carbon dioxid recorded as having been yielded by 10 grams of the soils is considerably larger than one-fifth of the quantity obtained from the 50-gram portions of the same soils. To explain this anomaly further investigatory work would be necessary. The above results are far from being satisfactory to me, and I intend to give the method a more thorough trial in the near future."

In the experience of other analysts the use of more than 10 or 15 grams of soil is unsatisfactory because of the larger bulk of substance in the flask, the greater amount of acid, the longer time required for completing the reaction, and the greater difficulty in removing all of the carbon dioxid from the acid solution. The lower results obtained by Charron on the 50-gram quantities of soil could probably be accounted for by the less complete removal of the carbon dioxid from the acid solution.

Supplementary Report by A. M. Peter.

As supplementary to the present report, the associate referee would present the following results and comments from A. M. Peter, of the Kentucky station, received after the other data had been compiled:

Some determinations of carbon dioxid have been made on the two official soil samples by Graham Edgar and H. B. Sanders, of the Kentucky station, by the method suggested for trial.

Sample No. 1, using 10 grams of soil, gave 0.074 and 0.080 per cent of carbon dioxid.

Sample No. 2, using 10 grams of soil, gave 0.026 and 0.025 per cent of carbon dioxid; using 20 grams of soil, 0.0225 per cent; using 40 grams of soil, 0.025 per cent; using 20 grams of soil with the addition of 0.05 gram of calcium carbonate gave 0.137 per cent of carbon dioxid.

These determinations seem quite satisfactory and the method is easy of manipulation and fairly rapid.

By using two potash bulbs, about three determinations could be made in an hour. The apparatus can be improved, however, by some slight modifications. The evolution flask in the Knorr apparatus is larger than is necessary for soil and a 100 cc flask was substituted, proving entirely satisfactory, even with as much as 40 grams of soil. Also, the inlet tube for the acid should not be drawn or bent as in the Knorr apparatus, since it is then apt to get stopped with soil. We also prefer rubber joints and used them in most of the work, the ground-glass joints being liable to break. A large cylinder filled with soda lime and connected by a rubber tube with the air-inlet tube of the apparatus was added, and was more satisfactory than depending on the small soda-lime tube to remove all carbon dioxid from the air that is drawn into the apparatus. In the Knorr apparatus the soda-lime tube is very small and the opening is situated in the draft from the Bunsen burner, which, of course, contains much more carbon dioxid than air ordinarily does; in the first part of the work, where only a small tube was used, irregularities were observed which were attributed to carbon dioxid getting by the soda lime.

A number of duplicate determinations were made by this method on samples in our regular soil work and these are also reported to show how close the duplicates run. Most of these determinations were made on soil that had been sifted through a 2-mm meshed sieve, but if the percentage of carbon dioxid was supposed to be large, the samples were more finely ground. For example, in sample No. 334, which was quite exceptional in containing small particles of limestone, concordant results were not obtained until the sample was finely ground. Determinations were made on 20 grams of soil in all except the two determinations on No. 334, in which 5 grams were used.

Duplicate determinations of the percentage of carbon dioxid in soils (Edgar and Sanders).

Number.	First determination.	Second determination.	Number.	First determination.	Second determination.
25223	0.019	0.022	547	0.028	0.028
25224	.077	.080	287	.016	.018
25225	.045	.049	134	.102	.104
25226	.044	.046	407	.052	.058
25227	.047	.051	401	.046	.056
25228	.098	.108	402	.028	.032
25229	.061	.065	405	.030	.033
25230	.054	.058	406	.013	.016
19	.150	.160	415	.045	.052
169	.123	.133	416	.030	.035
513	.229	.232	440	.045	.049
38	.020	.028	441	.030	.037
384	.010	.014	470	.050	.055
475	.013	.017	471	.020	.024
122	.026	.028	544	.045	.047
337	.044	.047	545	.025	.027
538	.030	.034	546	.022	.023
334[a]	1.914 1.997	2.080	562	.023	.028
			563	.019	.019
82	.033	.035	551	.045	.049
574	.036	.040	552	.036	.038
36	.143	.154			
378	.129	.152	Average	.0952	.1038

[a] Determination made on 5 grams of soil.

INTERPRETATION OF SOIL ANALYSES WITH RESPECT TO PHOSPHORIC ACID.

By G. S. Fraps.

The Texas experiment station has for three years been engaged in a study of the phosphoric acid of the soil, conducted by work in the laboratory and by pot experiments. It is my object to present merely an outline of some of the conclusions reached, the details to be published later.

The behavior of various mineral phosphates toward weak solvents has been studied, and we find that the phosphates of lime are easily dissolved in fifth-normal nitric acid, and so, as a rule, are the normal ferrous or ferric and aluminum salts, while the basic salts of iron and aluminum are only very slightly soluble. It has been concluded that the phosphoric acid dissolved from a natural soil, in excess of 9 parts per million, generally comes from the phosphates of lime. There may be soils which contain easily soluble non-basic phosphates of iron and aluminum, but it appears that such soils are exceptional. As a rule, then, the easily soluble phosphoric acid comes from phosphates of lime.

Now, it does not follow that soils containing the same quantity of phosphates of lime will react in the same way toward phosphatic fertilizers. The phosphates may differ in value in different soils. To take an extreme case, a soil receiving equal amounts of phosphoric acid in acid phosphate and phosphate rock will react the same toward the solvent, but very differently to plants. And the same is true for various phosphates of lime used for fertilizing purposes. There is no solvent which shows the relative value of all phosphates outside of the soil in a pure state, and it is not to be expected that a solvent will bring to pass in the soil what we can not effect with the phosphates themselves.

The phosphates which are dissolved by a solvent may be on the outside of the soil particles and exposed to plant roots, or they may be within the soil particles. In a calcareous soil a large portion of the phosphates of lime may be inclosed within particles of carbonate of lime and inaccessible to plants. Furthermore, the quantity of acid consumed during the digestion of the soil is to some extent a measure of the lime and magnesia dissolved. If 50 per cent of the acid is consumed, that means that about 5 per cent of carbonate of lime and magnesia, or compounds corresponding to these, have been dissolved. That is to say the dissolved phosphates may be distributed through 100,000 pounds of this soil per acre-foot. To what extent they are so distributed, to be presented to the plant by slow processes of weathering, is not known, but we must consider carefully the complication which this fact introduces into our soil analysis. To this fact is probably due the durability of limestone soils.

The phosphoric acid dissolved from a soil in excess of 8 parts per million is, in most cases, present as phosphate of lime. But a distinction must be made between the phosphate which goes into solution and that which is removed in the extract. A portion of the dissolved phosphoric acid is withdrawn from solution by the soil, and this may vary from 0 to 97 per cent. In the case of one soil to which 200 parts per million of phosphoric acid was added we extracted about 14. The soil with no addition gave about 8 parts, making a loss of about 194 parts per million, or 97 per cent. This is an extreme case. Yet who, considering these facts, would venture to say how much phosphate of lime this soil contains? Several soils studied absorbed over 50 per cent of the

phosphoric acid presented to them in acid solution. The absorptive power of the soil, then, must be taken into account.

Reducing it to its lowest terms, the analysis of a soil with fifth-normal nitric acid amounts to this:

Knowing the quantity of phosphoric acid extracted by the solvent, and the absorptive power of the soil for phosphoric acid, estimate how much phosphate of lime is present in the soil. Then, knowing the amount of acid consumed, consider to what extent this phosphate is distributed within the mass of the dissolved material and to what extent it is exposed to the roots of the plants. Having estimated the amount of exposed phosphate of lime, we have next to inquire how much of it is necessary to make a soil fertile. What conditions affect the rate and the quantity of phosphoric acid which these phosphates give up? Then the probable value of the basic ferric and aluminum phosphates present must be considered, and whether or not organic phosphates are in the soil. Having considered all these questions, we will be in a position to interpret the analysis of a soil with fifth-normal nitric acid.

I do not wish to discredit this method, but merely to point out the extreme complexity of soil analyses and the problems which must be solved in connection with it which are being studied at the Texas experiment station. I believe that the fifth-normal nitric acid method offers an excellent means of judging soils of similar types or characteristics. Good results have been obtained with it in our pot work and in studying the needs of various types of Texas soils.

METHODS FOR THE DETERMINATION OF THE NITRIFYING AND AMMONIFYING POWERS OF SOILS.

By F. L. Stevens and W. A. Withers.[a]

Nitrate nitrogen is generally believed to be the most readily available and most valuable form of nitrogen for plants If this view be true, even in part, methods for measuring the ability of various soils to produce nitrate nitrogen (to nitrify) are needed, since on the nitrifying power of the soil would depend to some extent, possibly to a large extent, its productivity. To determine nitrifying power, to recognize deficiencies in nitrifying power, to ascertain the cause of such deficiencies when they exist, and to find means of correcting them all require quantatitive studies of this factor of soil fertility. To make quantitative determinations of nitrifying power that shall be of broad utility and general value, methods which may be regarded as standard must be devised and their trustworthiness recognized. The biological equilibrium in soils is one of utmost instability, and variations in methods are likely to lead to great discrepancies in results. These facts[b] have been constantly forced upon us during the investigations of the past few years and have led to the consideration of the question of uniform methods, the results of which are presented herewith.

Nitrifying Indices.

Three conditions to be recognized in considering the nitrifying ability of a soil are:

1. The nitrifying organisms present.

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[a] Assisted by J. C. Temple, W. A. Syme, J. K. Plummer, and P. L. Gainey.

[b] Stevens and Withers, Studies in Soil Bacteriology, 1. Nitrification in Soils and in Solutions. Centralbl. f. Bak., **23** (2) : 355–373.

2. The physical and chemical fitness of the soil for the proper functioning of these organisms.

3. The nitrifying efficiency of the soil and the organisms existing in it.

NITRIFICATION INOCULATING POWER (N. I. P.).

The first index may be called the nitrification inoculating power, which may be abbreviated as N. I. P. It recognizes only the factor of the live organisms present, taking no account of the fitness or unfitness of the soil for their activity. It does not regard bacterial species, but merely the complex present in the soil at the time it is tested.

Theoretically the N. I. P. may be high in a soil in which, owing to adverse chemical or physical conditions, no nitrification really occurs. Theoretically nitrifying bacteria may be present in goodly numbers in a soil possessing physical and chemical conditions favorable to rapid nitrification, yet no nitrate will appear, owing to the presence of other species of bacteria or of substances which either inhibit the action of the nitrifiers or destroy the nitrate which they produce, so that nitrate does not appear as a final product. N. I. P. considers only the efficiency of the organisms present to give nitrate as a final product under circumstances favorable to their growth.

NITRIFYING CAPACITY (N. C.).

The second index, fitness of the soil as regards factors other than its content of living things, i. e., its capacity to support nitrification provided proper organisms be present, may be designated as its nitrifying capacity, abbreviated as N. C. This index regards only the non-living factors. Theoretically a soil may be of high N. C., but still fail to nitrify, owing to lack of proper organisms, i. e., to lack of proper N. I. P. Theoretically a soil may be of low N. C. yet show high N. I. P. N. C. will, on final analysis, be found to depend upon physical conditions and chemical composition, including water content.

NITRIFYING EFFICIENCY (N. E.).

The third index may be designated as the nitrifying efficiency of the soil, abbreviated N. E., which regards the efficiency of the soil as a whole to produce nitrates as a final product. N. E. may be low, owing to lack of N. I. P. or to lack of N. C., or both. N. E. will be high if there is high N. I. P. associated with high N. C.

The proper determination of N. E. will show whether a given soil is in a normal vigorous nitrifying condition; if such is not the case, the determination of N. C. and N. I. P. will show whether it is the bacterial or non-bacterial factors which are at fault and may lead the way to the correction of existing defects.

CONDITIONS FOR DETERMINING THESE INDICES.

To make each of these indices of the greatest value the determination must be made as nearly as possible under the conditions that normally obtain in the field. Field conditions are highly variable from season to season, even from hour to hour, and may never be twice exactly alike. They can, therefore, only be approximated. As the soil temperature is extremely variable, sometimes above, sometimes below the optimum for nitrification, it seems best to employ the optimum temperature as a standard.

Since the water relation is of dominating importance[a] the amount of water in the soil cultures should imitate the natural condition of the soil that is to

[a] Stevens and Withers, loc. cit.

be tested. If this soil is normally saturated or is under water, or swampy, the cultures should provide these conditions. It is difficult to determine the degree of saturation which best imitates the condition of a normal well-drained field. During, and shortly after a rain, it is saturated, but it dries rapidly and until another rain occurs is considerably below saturation. Since, however, the greater part of the nitrification that does occur must take place during a period approximating the optimum water content, it seems best to use, as nearly as is practicable, the optimum degree of saturation for all soils other than soils normally saturated, as mentioned above.

Methods for Standard Analysis.

Based on preliminary experiments and on results previously reported, the following rules for determining the nitrifying efficiency, nitrification inoculating power, and nitrifying capacity of soils have been formulated.

To determine nitrifying efficiency of soils.

Screen the soil to be tested through a wire sieve of from 3 to 4 mm mesh (opening), to remove stones, sticks, and coarser matter and get the soil in a good condition to manipulate; then determine its saturation capacity.

To do this 50 grams of soil, the present saturation of which is known, are placed on a porcelain plate in a carbon filter, a measured quantity of water (a little more than is necessary to saturate the soil) is added, and the effluent is poured upon the soil and allowed to drain through again. This operation is repeated twice to insure complete saturation. The amount of water taken up by the soil is determined by measuring the final effluent and subtracting it from the measured quantity originally employed. Allowance is made for water already in the soil. Weigh live soil equal to 400 grams of dry soil (dried two hours at 110° C.) into a 500 cc erlenmeyer; add 240 mg of nitrogen as sterilized ammonium sulphate solution, with proper precautions to prevent the introduction of germs extraneous to the soil (i. e., using sterile sieves, containers, ladles, washing the hands with nearly surgical care, etc.); add sterile water sufficient, together with that already in the soil and in the ammonium sulphate solution, to make it closely approximate a two-thirds saturation; plug loosely with cotton and incubate at from 30° to 35° C. for four weeks.

Set up in duplicate and with two control flasks; i. e., 400 grams of the same soil in the same condition but without added nitrogen. Make up water loss weekly in all flasks. Determine the amount of nitrite and nitrate present in each flask at the end of the incubation period, and report in terms of the element nitrogen.

Subtract the average of the nitrite and nitrate nitrogen found in the two control flasks to give the net *nitrite and nitrate nitrogen* produced by organisms in the soil in question.

Express the nitrifying efficiency as a coefficient equal to the per cent of the original 240 mg of added nitrogen that is represented by the net nitrite and nitrate nitrogen found.

When determining the nitrifying efficiency for soils normally saturated or immersed, test the soils saturated instead of two-thirds saturated.

Discussion of nitrifying efficiency method.

To strictly reflect field conditions, the humidity should be that of the field. Field conditions, however, are very variable and such nitrification as does occur takes place mainly at a nearly optimum moisture. A two-thirds saturation is therefore adopted as a standard condition for normally drained agricultural soils and, of course, a condition of complete saturation for all soils that are normally saturated. Since soils vary in their optimum moisture relation, it would be still better to use the optimum for each separate soil studied. This is, however, manifestly impossible except in the case of an exhaustive research on special soils.

The temperature suggested is near the optimum for most nitrifying bacterial complexes, and is adopted for substantially the reasons leading to the adoption of the optimum saturation.

The time, four weeks, nearly one-third of a crop-growing season, is ample to give easily measured results in cases of soils in good nitrifying condition. Very poor nitrifiers would give different records with longer time; but this would retard work greatly, probably without affecting conclusions materially.

Aeration is probably better under these test conditions than in the field, but not so different as to materially affect results.

That some of the nitrate formed in the soils is not recovered by analysis is certain. This is the chief objection to the use of soils as a medium, but in view of the complete inadequacy of solutions as a medium there is no escape from the dilemma, and soils must be used.

TO DETERMINE NITRIFICATION INOCULATION POWER OF SOILS.

Make a bacterial suspension from the soil to be tested, using 100 grams of soil to 200 grams of sterile distilled water, shake three minutes, sediment for five minutes, and use the supernatant fluid. All work must be done aseptically. As a medium use the equivalent of 400 grams of dry standard soil, sterilized, two-thirds saturation, less the water in the ammonium sulphate and bacterial suspension, with 240 mg of nitrogen added as ammonium sulphate. After inoculation with 75 cc of the bacterial suspension from the soil to be tested, culture in Erlenmeyer flasks as for the determination of N. E.

Make the determination in duplicate and determine the nitrite and nitrate nitrogen present at the end of four weeks. From the average of the two flasks subtract nitrite and nitrate nitrogen equal to that in the culture at the beginning, if any, to secure the net nitrite and nitrate nitrogen produced.

Express N. I. P. as a coefficient equal to the per cent of original nitrogen changed to nitrite and nitrate nitrogen under these standard conditions in standard time. When determining N. I. P. for soils normally saturated, use saturated cultures.

DISCUSSION OF NITRIFICATION INOCULATION POWER METHODS.

An artificial soil of high N. C., which might serve as a universal standard, is desired, but our attempts to construct such an artificial medium have failed utterly. With the use of a different standard soil somewhat different results would be obtained, but the results given by the method here outlined approach the truth more nearly than the earlier methods employed and closely approximate the truth. No one medium can reveal the whole truth.

TO DETERMINE NITRIFYING CAPACITY OF SOILS.

The soil to be tested shall be prepared as for the determination of N. E., then sterilized, inoculated, and subsequently treated as for the determination of the N. I. P., using the same inoculum at the same time in parallel cultures in standard soil. All cultures must be made in duplicate with controls.

The N. C. is determined by dividing the coefficient given by the soil to be tested by the coefficient of the standard soil.

If it were possible to maintain a standard inoculum, the N. C. could be directly determined, but means of doing this have not yet been devised. It is recognized that the use of a different inoculum would lead to somewhat different results and that tests with any one inoculum can not reveal the whole truth.

Standard Method for Determining Ammonification.

For the reasons given regarding nitrification, it is believed that the ends of soil bacteriology will be furthered by the adoption of similar indices regarding the soil factor of ammonification. We therefore propose the terms ammonifica-

tion efficiency (A. E.), ammonifying inoculating power (A. I. P.), and ammonifying capacity (A. C.) to be determined in the following ways:

TO DETERMINE AMMONIFICATION EFFICIENCY.

The sample is to be prepared and tested as for N. E., with the exception that 200 grams of soil are used and 120 mg of nitrogen are added as cottonseed meal instead of as ammonium sulphate; incubation lasts for seven days, and the analysis is made for ammonia.

TO DETERMINE AMMONIFYING INOCULATING POWER.

The sample is prepared and tested as for N. I. P., except that nitrogen is added as cottonseed meal, medium is 200 grams, inoculum 25 cc, incubation lasts for seven days, and analysis is made for ammonia.

TO DETERMINE AMMONIFYING CAPACITY.

The sample is prepared and tested as for N. C., medium 200 grams, the inoculum 1 cc of a pure culture of *B. subtilis* twenty-four hours old in standard beef bouillon at incubator temperature, which culture is inoculated with one oese from another culture. This inoculum must be frequently standardized against standard soil and corrections made for any change from its original ammonifying power.

A RAPID METHOD FOR THE DETERMINATION OF TOTAL POTASSIUM IN SOILS.

By O. M. Shedd.

The method herein proposed for the determination of total potassium in soils has been published by the writer in the May number of the Journal of Industrial and Engineering Chemistry. Since that publication the method has been further tested in this laboratory with the assistance of S. D. Averitt, and also by E. Van Alstine and J. B. Park, of the Illinois station; C. K. Francis, of the Missouri station; and L. T. Bowser, of the Ohio station, to all of whom the author acknowledges his indebtedness.

The soils worked upon were the two samples sent out this year by the referee on soils and the work as outlined by him consisted in determinations of potassium by the J. Lawrence Smith method and by Pettit and Ystgard's modification of that method. The association work, therefore, was a comparison of the regular Smith and modified Smith methods and the cooperation requested by the writer was that a determination or two be made on the same samples by the new method in order to compare it with the Smith method in the hands of different analysts.

The new method briefly described is a combination of the J. Lawrence Smith [a] method with the cobalti-nitrite method of W. A. Drushel,[b] the potassium being brought into solution by the former method and determined by the latter without previous separation of the calcium. The fusion is made by the Smith method, slaked with water, filtered, and washed. The whole filtrate, or an aliquot, is slightly acidified with acetic acid, concentrated in a large dish or casserole, an excess of sodium cobalti-nitrite reagent added, and evaporated to a pasty condition on the water bath until the residue is just dry on cooling.

[a] Fresenius, Quantitative Analysis, Amer. ed., p. 426.

[b] Adie and Wood, J. Chem. Soc., 1900, **77**: 1076; Drushel, Amer. J. Sci., 1907, **24**: 433; Chem. News, 1908, **97**: 124.

The residue is taken up with sufficient water to dissolve the excess of reagent, filtered through asbestos in a Gooch crucible, washed with water, or preferably a one-half saturated solution of sodium chlorid. The asbestos pad and crucible are put back in the same dish, an excess of tenth-normal potassium permanganate is added, diluted to eight or ten times its volume and kept just under the boiling point for from six to eight minutes or until the solution darkens and manganese hydroxid separates.

Five to twenty-five cubic centimeters of sulphuric acid (1 : 7) are then added and the whole allowed to stand a few minutes longer to complete the oxidation. An excess of tenth-normal oxalic acid is then run in and the solution is allowed to stand at the same temperature until decolorized, when it is titrated to color with permanganate. The total volume of permanganate solution used, less that equivalent to the oxalic acid added, gives the amount used in oxidizing the cobalti-nitrite, and this multiplied by the appropriate factor gives the weight of potassium present. One cubic centimeter of tenth-normal potassium permanganate solution is equivalent to 0.000711 gram of potassium or 0.000856 gram of potassium oxid.

In the original article attention was called to possible sources of error, and in all subsequent work, by watching these points, no trouble was experienced in obtaining concordant results either on soils or on blank determinations. On the other hand, the low results obtained at that time the writer attributes to the imperfect decomposition of the silicates caused by the lack of sufficient heat in making the fusion, as will be shown by a comparison of the results given in the tables.

In order to eliminate these differences from the comparison of the gravimetric with the volumetric determination, the plan was adopted of dividing the solution from the Smith fusion into two equal parts, in one of which the potassium was determined by the platinum method and in the other by the cobalti-nitrite method.

In the first series of experiments the fusion was made by using the full heat of one Bunsen burner without any chimney, but in a place free from draft. The crucible was put in a hole, cut to fit, in an asbestos board, so that the top would be comparatively cool, thereby avoiding loss of chlorids by volatilization. The results obtained by this method of heating are given in Table 1.

TABLE 1.—*Determination of potassium (water-free basis) in soil No. 2, with fusion by heat of one Bunsen burner, without chimney.*

J. L. Smith method.[a]	Cobalti-nitrite method.[a]
Per cent.	*Per cent.*
1.585	1.556
1.495	1.428
1.512	1.475
1.347	1.330
1.588	1.699
1.453	1.508
1.475	1.578
1.553	1.600
Average . 1.501	1.522

[a] Same fusion, solution divided.

It will be noted that each volumetric result compares very well with its corresponding gravimetric result, but that between the results from different fusions there are large variations. The natural conclusion was that most of the results were low, due primarily to insufficient heat in making the fusions,

and in all of the writer's subsequent work the Bunsen burner was surrounded by a clay cylinder in the form of a chimney, so as to keep the heat on the crucible.

The crucible in the asbestos board, as before, was placed just high enough above the top of the cylinder to give a good draft, while at the same time all of the heat was kept on the lower part of the crucible. The blast lamp was also used in making a number of the fusions, and as a rule slightly higher results were obtained. To show what an important factor the heat employed in making the fusion is, the results obtained by the Smith method on soil No. 2 in this laboratory, as given in Tables 1 and 3, should be compared. First, where the Bunsen burner alone was used without any protection, the average of eight determinations gave 1.501 per cent of potassium, as given in Table 1. But when the Bunsen burner was protected by a clay cylinder, so as to keep the heat on the crucible, the average of 13 determinations gave 1.679 per cent of potassium, as shown in Table 3. And finally, when the blast lamp was used in making the fusion, the average of 10 determinations, omitting one which was evidently low, gave 1.701 per cent of potassium.

The writer, therefore, would advocate using the heat of the blast lamp or its equivalent in making fusions by the Smith method, on the ground that more uniform and more accurate results on the total amount of potassium present are thus obtained, provided that proper precautions are taken to keep the upper part of the crucible cool. This is in line with the suggestions made by Hillebrand,[a] who advocates the use of nearly the full heat from two Bunsen burners, and of Wiley,[b] who recommends the use of the blast lamp.

The cooperation results obtained on soil No. 1 are given in the following table:

TABLE 2.—*Determination of potassium (water-free basis) in soil No. 1.*

Analyst.	J. L. Smith method.[a]	Cobaltinitrite method.[a]
	Per cent.	*Per cent.*
E. Van Alstine, Illinois	1.485	1.440
	1.445	1.284
Average	1.465	1.362
J. B. Park, Illinois	1.388	1.688
	1.502	1.563
	[b] 1.578	[b] 1.670
	[b] 1.569	[b] 1.679
Average	1.509	1.650
O. M. Shedd, Kentucky	1.527	1.526
	1.491	1.483
	1.535	1.521
	1.490	1.474
	1.527	1.518
Average	1.514	1.504
General average	1.503	1.531

[a] Same fusion, solution divided.
[b] Separate fusion.

As the soil reported on in this table is the same as the No. 2 sample used by the writer in former work, the results obtained then are of interest for

[a] U. S. Dept. Interior, Geological Survey Bul. 305, p. 145–146. The Analysis of Silicate and Carbonate Rocks, by W. F. Hillebrand.

[b] Principles and Practice of Agricultural Analysis, 2d ed., 1906, vol. 1, p. 424.

comparison. At that time the average of four determinations by the Smith method gave 1.460 per cent of potassium, and the average of nine by the cobalti-nitrite method gave 1.490 per cent. This seems to verify the statement previously made that the uniform low results then obtained were due to the lack of heat employed in making the fusion. The results obtained on soil No. 2 are given in Table 3.

TABLE 3.—*Determination of potassium (water-free basis) in soil No. 2.*

Analyst.	J. L. Smith method.[a]	Cobalti-nitrite method.[a]
E. Van Alstine, Illinois	[b] 1.622	[b] 1.658
	[b] 1.578	[b] 1.637
	[b] 1.587	[b] 1.659
	[b] 1.509	[b] 1.681
	1.586	.....
Average	1.576	1.659
J. B. Park, Illinois	[b] 1.641	[b] 1.605
	[b] 1.605	[b] 1.591
	1.630	1.621
	1.635	1.578
Average	1.628	1.599
C. K. Francis, Missouri	1.700	1.733
	1.576	1.801
	1.678	1.815
Average	1.651	1.783
S. D. Averitt, Kentucky	1.670	1.588
	1.660	1.581
	1.718	1.574
	1.738	1.574
	1.696	1.574
	1.670	.....
	[c] 1.628	.....
	[c] 1.725	.....
	1.689	.....
	1.689	.....
	1.667	.....
	[c] 1.702	.....
Average	1.688	1.578
O. M. Shedd, Kentucky	[b] 1.659	[b] 1.744
	[b d] 1.682	[b] 1.716
	[b] 1.681	[b] 1.702
	[b d] 1.672	[b] 1.702
	[b c d] 1.717	[b c] 1.773
	1.639	1.681
	1.652	1.709
	[c] 1.659	[c] 1.664
	[c] 1.675	[c] 1.636
	[c] 1.694	[c] 1.650
	[c] 1.646	.....
	[c] 1.678	.....
	[c] 1.752	.....
	[c] 1.746	.....
	[c] 1.730	.....
Average	1.684	1.698
General average	[e] 1.661	[f] 1.663

[a] Separate fusion, except when otherwise stated.
[b] Same fusion, solution divided.
[c] Blast lamp used in making fusion.
[d] Modified J. L. Smith method.
[e] Thirty-six determinations.
[f] Twenty-six determinations.

L. T. Bowser, of the Ohio station, reports that he failed to get satisfactory results by either method on soil No. 1. By the Smith method, on soil No. 2, he obtained 1.630 per cent and 1.590 per cent of potassium, and by the cobalti-

nitrite method on the same soil, from a separate fusion, he obtained 1.460 per cent. Mr. Bowser also reports that he has always found Drushel's method unsatisfactory, this case proving no exception. These results were received too late to be included in the general averages, and, as they do not make any appreciable change, a revision of Table 3 was not thought necessary.

On the whole, there is a close agreement between the two methods, considering that one of them was new in the hands of most, if not all, of the analysts who cooperated in the work. It has been the writer's experience that the new method will agree as well with the regular J. Lawrence Smith method as will the modified Smith method. To illustrate: The work of this year by the writer, using the three methods, gave the following results on soil No. 2: J. Lawrence Smith method, average of 12 determinations, gave 1.684 per cent of potassium; the modified Smith method, average of 14 determinations, gave 1.625 per cent; and by the cobalti-nitrite method, the average of 10 determinations gave 1.698 per cent of potash. These results also agree very closely with the general averages obtained for this year by the analysts using the two methods in the association work. The general averages, as reported by the referee on soils, show 1.677 per cent of potassium by the J. L. Smith method and 1.629 per cent by the modified Smith method.

The new method has a decided advantage over the others now in use, inasmuch as it requires no expensive reagents and only about one-half of the time to make a determination, which makes it very desirable where many samples are to be analyzed at the same time.

THURSDAY—AFTERNOON SESSION.

APPOINTMENT OF COMMITTEES.

The following committees were appointed by the president:

Committee on amendments to the constitution: B. L. Hartwell, J. P. Street, and G. S. Fraps.

Committee on resolutions: F. W. Woll, J. M. Bartlett, and C. S. Cathcart.

Committee on nominations: B. B. Ross, A. J. Patten, and W. B. Ellett.

REPORT ON INSECTICIDES.

By C. C. McDonnell, *Referee.*

Plan of Work.

The work on methods for the examination of insecticides has been a continuation of the studies made last year along the following lines:

(1) A comparison of the provisional Methods I and II for total arsenious and arsenic oxids in London purple, given in Bureau of Chemistry Bulletin 107, Revised, and a modified method for total arsenic oxid proposed by the present referee and submitted last year.

(2) A continuation of the study of the provisional method for the analysis of lead arsenate proposed by Haywood.

(3) A further study of the precipitation method for soda lye, using fifth-normal acid instead of half-normal, also with and without removal of the barium carbonate precipitate before titration.

(4) Determination of formaldehyde in strong solution by the provisional hydrogen peroxid method and in dilute solutions by this and the cyanid method.

(5) Further study of the Avery method for the determination of total sulphur in sulphur dips.

Samples were sent to chemists in 15 laboratories, who volunteered to cooperate in the work. Owing to pressure of other duties and the early date of the meeting of the association, reports were received on all or a portion of the samples from only six laboratories, representing the work of nine chemists.

London Purple.

Methods I and II for arsenious oxid in London purple are given in Bulletin 107, Revised, pages 28 and 29.

Total arsenious oxid (As_2O_3).

Analyst.	Arsenious oxid (As_2O_3).		Analyst.	Arsenious oxid (As_2O_3).	
	Method I.	Method II.		Method I.	Method II.
	Per cent.	*Per cent.*		*Per cent.*	*Per cent.*
O. M. Shedd, Lexington, Ky.	.....	10.40	W. B. Pope, Washington, D. C.	10.78	10.27
	.....	10.44		10.87	10.36
	.....	10.50		10.78	10.36
R. J. Davidson, Blacksburg, Va.	10.76	10.32		10.78	10.27
	10.84	10.44	C. C. McDonnell, Washington, D. C.	10.76	10.36
H. V. Tartar, Corvallis, Oreg.	.....	10.95		10.72	10.48
	.....	10.95	Average.....	10.79	10.47

Method I for total arsenic oxid is given in Bulletin 107, Revised, page 28, and Method II on page 29. Method III, proposed by the referee and tried last year, is as follows:

Referee's modification of method for total arsenic oxid (method iii).

Weigh 2 grams of the sample, transfer to a 250 cc graduated flask,[a] and add 5 cc of concentrated nitric and 20 cc of concentrated sulphuric acid. Place on a hot plate or over a low flame and heat to boiling. After ten or fifteen minutes add powdered sodium nitrate, in small quantities at a time, until all organic matter is destroyed and the solution is colorless. Cool, add about 50 cc of water (to decompose any nitro-sulphuric acid formed) and heat again until the nitric acid fumes are all expelled. Cool, make up to mark with distilled water, mix thoroughly, filter through a dry filter, and determine arsenic oxid in 50 cc of the filtrate (0.4 gram).

Transfer this 50 cc portion to a 400 cc Erlenmeyer flask, dilute to 100 cc with water, add from 2 to 3 grams of potassium iodid, heat to boiling and evaporate to about 40 cc (not less). Cool, dilute to 150 to 200 cc, and add approximately tenth-normal sodium thiosulphate just to disappearance of color caused by the free iodin. In case the solution is slightly colored from organic matter or from any cause other than free iodin, add the thiosulphate until it is nearly colorless, then add a few drops of starch paste, and continue adding the thiosulphate slowly until the blue color just disappears. The exact end point can easily be obtained in this way. Neutralize immediately with sodium carbonate, make slightly acid with dilute sulphuric acid, and when all lumps of sodium carbonate are dissolved, add sodium bicarbonate in considerable excess. Titrate with twentieth-normal iodin solution, using starch solution as indicator in the usual way. Subtracting from this the number of cubic centimeters of

[a] Owing to the high boiling point of concentrated sulphuric acid, it has been found more satisfactory to make the digestion in a Kjeldahl nitrogen flask and then transfer to the graduated flask.

iodin solution corresponding to arsenious oxid, as obtained by Method I, gives the number of cubic centimeters of iodin solution corresponding to the arsenic oxid (As_2O_5) in 0.4 gram of the sample, and from this figure the per cent may be calculated.

Total arsenic oxid (As_2O_5).

Analyst.	Method I.	Method II.	Method III.
	Per cent.	*Per cent.*	*Per cent.*
O. M. Shedd, Lexington, Ky	.....	28.93	30.97
	.....	30.14	30.91
	.....	29.60	.....
R. J. Davidson, Blacksburg, Va	29.52	29.77	30.53
	29.68	29.67	30.57
H. V. Tartar, Corvallis, Oreg	.....	28.24	30.25
	.....	27.88	30.25
W. B. Pope, Washington, D. C	30.02	29.16	30.02
	30.32	28.77	30.22
	30.52	28.50	30.29
	30.52	28.50	30.09
C. C. McDonnell, Washington, D. C	30.19	29.71	30.34
	30.48	30.10	30.12
	.....	.....	30.17
	.....	.....	30.55
Average	30.16	29.15	30.38

O. M. Shedd comments as follows: "Method III for arsenic oxid in London purple is very good, but I think the other method cited, where hydrochloric acid is used without concentrating, is open to objection on account of the coloring matter present, which interferes with the end point in the elimination of free iodin with thiosulphate and also in the final titration with iodin."

H. V. Tartar says: "The referee's modification of the determination of total arsenic oxid in London purple seems to be a decided improvement over the provisional method."

The results on total arsenious oxid agree very well, Method II giving, as in past years, slightly lower results.

The results on arsenic oxid show much closer agreement than has been the case in the past. The average found by Method III is slightly higher than that given by Method I, and the average by Method II is 1 per cent lower than by Method I. Method II has in practically all cases in the past given lower results than Method I, the difference sometimes amounting to several per cent. This has been attributed to the fact that on making alkaline with sodium carbonate to remove a portion of the coloring matter, some arsenic compound, probably calcium arsenate, was precipitated at the same time. In order to determine the effect of a larger amount of calcium 2 grams of calcium chlorid were added to 2 grams of the London purple and the determination of arsenic carried out according to Method II, with the following results:

	Per cent.
Arsenious oxid (As_2O_3)	10.25
	10.32
Arsenic oxid (As_2O_5)	28.46
	28.08

On washing the precipitate produced by sodium carbonate, dissolving in hydrochloric acid, and passing in hydrogen sulphid considerable arsenic was precipitated.

It will be seen that arsenious oxid and arsenic oxid are both lower in this case, which seems to show conclusively that the main source of error lies

here, and from the results obtained in the past the method can not be relied upon.

Method I has given satisfactory results, but for total arsenic oxid the presence of the dye is quite troublesome, and some experience with the method is required before one can be certain of his results.

The difference between the highest and lowest determination reported by Method III is less than 1 per cent. According to the experience of the writer and the reports of others who have used it, this method is easy of manipulation and gives good results. The main objection to it is that for the determination of arsenious oxid a separate weighed portion must be used.

Lead Arsenate.

The methods used for lead arsenate were proposed by Haywood in 1906 and adopted provisionally by the association at its meeting in 1907. They may be found in Bureau of Chemistry Bulletin 107, Revised, page 239.

The sample of lead arsenate sent out for the work was made by the referee from C. P. disodium arsenate and lead acetate.

Lead arsenate.

Analyst.	Moisture.	Total arsenic oxid (As_2O_5.)	Total lead oxid (PbO).	Water-soluble arsenic oxid (As_2O_5).	Water-soluble lead oxid (PbO).	Water soluble, exclusive of arsenic and lead oxids.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
O. M. Shedd, Lexington, Ky	0.15	31.25	66.54	0.31	0.00	0.34
	.15	31.21	66.63	.31	.....	.34
	.....	31.14	66.58	.....	.....	.....
R. J. Davidson, Blacksburg, Va	.....	30.77	.....	.....	.....	.....
	.....	30.80	.....	.....	.....	.....
C. E. Bradley, Corvallis, Oreg	.08	31.00	66.67	.....	.....	.....
	.09	31.06	66.59	.32	.00	.90
H. V. Tartar, Corvallis, Oreg	.07	30.96	66.67	.14	.....	1.06
	.08	30.96	66.60	.14	.00	1.16
A. G. Spencer, Ottawa, Canada	.08	31.20	66.59	.19	.02	.40
	.07	31.24	66.59	.23	.02	.....
	.08	31.27	66.49	.27	.02	.30
	.....	31.20	.....	.23	.04	.....
W. B. Pope, Washington, D. C	.10	30.93	66.10	.46	.....	.....
	.11	30.78	66.23	.46	[a] 00	.64
	.....	30.93	66.01	.....	.....	.80
	.....	30.78	66.14	.....	.....	.....
C. C. McDonnell, Washington, D. C	.11	30.87	66.43	.40	.....	.77
	.10	30.87	66.25	.40	[a] .00	.87
	.....	30.78	66.43	.....	.....	.....
	.....	30.87	66.49	.....	.....	.....
Average	.....	30.99	66.44	.30	.....	.....

[a] A precipitate weighing from 0.7 to 1 mg, consisting of oxid of iron, was obtained from 0.5 gram of material; no lead present.

C. E. Bradley says:

It is difficult to obtain the proper end point in removing the free iodin with thiosulphate after reduction with potassium iodid and sulphuric acid in the case of certain lead arsenates handled by us, a reddish yellow coloration persisting in the solution after the thiosulphate has been added in excess. Experiments have proven this to be true where dextrin is present in the original material. The use of a starch indicator is necessary in such cases.

H. V. Tartar suggests that 4 grams of the sample be used, and the solution made to 500 cc in order to obtain a sufficient volume for the determination of

total lead and arsenic oxids in duplicate. He also states that it was found more convenient and accurate to weigh the lead sulphate in a Gooch crucible.

The results on both total lead and arsenic oxids are good, the method for total arsenic oxid being easily carried out and accurate. The methods for total and soluble lead oxid will give accurate results on a pure lead arsenate, but for one containing impurities such as calcium salts, or any base whose sulphate is insoluble in alcohol of the strength designated, it will require some modification. In such cases the lead may be separated as sulphid by hydrogen sulphid or by igniting the original material with sodium carbonate and sulphur in a porcelain crucible. The referee does not consider duplicate determinations of much value unless made in duplicate throughout. For the filtration of the lead sulphate a porcelain Gooch crucible can be used to advantage particularly if a number of determinations are being made at the same time. The lead sulphate precipitate obtained sometimes decrepitates on being heated. For this reason the crucible should be kept covered during the ignition until all danger of loss from this cause is over.

Soda Lye.

Method I is the precipitation method given in Bulletin 107, Revised, page 31. Method II is the same as Method I, except that the titration for hydroxid is made in the presence of the barium carbonate precipitate.

The sample submitted was prepared by weighing into a bottle 3 grams of dry sodium carbonate C. P. and 17 grams of commercial sodium hydrate (Greenbank alkali) and sealing tightly. The analyst was directed to dissolve the entire content of the bottle in carbon dioxid-free water, make up to 1,000 cc and use 50 cc portions (1.0 gram sample) for the titrations. In the titration in the presence of the barium carbonate it was directed to add the acid slowly and stir vigorously, using fifth-normal acid in all cases, and to make correction for the amount of acid required to produce the tint titrated to with methyl-orange indicator, using the same volume of water as in the determination.

Cooperative results on soda lye by two methods.

Analyst.	Method I.		Method II.	
	Sodium hydroxid ($NaOH$).	Sodium carbonate (Na_2CO_3).	Sodium hydroxid ($NaOH$).	Sodium carbonate (Na_2CO_3).
O. M. Shedd, Lexington, Ky	80.06	17.52	80.74	16.61
	80.06	17.52	80.78	16.56
	80.06	17.52	80.78	16.56
			80.78	16.56
H. V. Tartar, Corvallis, Oreg	75.80	18.49	76.00	
	75.80	18.49	76.00	
A. T. Charron, Ottawa, Canada	78.90	17.27	79.41	16.59
	78.90	17.27	79.41	16.59
L. D. Havenhill, Lawrence, Kans.[a]	80.54	18.39	80.66	18.23
	80.38	18.60	80.66	18.23
			80.42	18.55
			80.02	19.08
			80.66	18.23
W. P. Pope, Washington, D. C	79.96	17.21	80.20	16.89
	79.88	17.32	80.28	16.78
C. C. McDonnell, Washington, D. C	79.97	17.21	80.38	16.67
	80.09	17.16	80.38	16.78
	79.96	17.23	80.30	16.78
Average	79.12	17.52	79.65	16.67

[a] Normal sulphuric acid used for total alkalinity; results not included in average.

O. M. Shedd says:

I am inclined to think that more correct results will be obtained if the barium carbonate is filtered, for when this is present the end point is not sharp and tends to give higher results. This is probably due to the action of the acid on the barium carbonate during the titration and to the fact that the barium carbonate tends to hold the pink color.

With one exception the results agree very well when the directions given were followed. Somewhat higher results are obtained on hydroxid and lower figures on carbonate when the titration is carried out in the presence of the barium carbonate precipitate, and the end point is more uncertain, particularly if there is much carbonate present. Little or nothing is gained so far as time is concerned, and the practice is not to be advised. By making to the mark after adding barium chlorid, allowing to settle, and pipetting off an aliquot portion, accurate results should be obtained if the necessary precautions are observed, the main point being that the barium chlorid must not be added in excess, only just enough for exact precipitation being used. Better results are also obtained by using a dilute standard acid, not stronger than half-normal.

Formaldehyde.

Method I (modified hydrogen peroxid) and Method II (cyanid) are given in Bulletin 107, Revised, page 33.

Two samples were sent out for analysis: No. 1, a strong solution to be analyzed by Method I, and No. 2, a dilute solution to be worked by both Methods I and II.

Cooperative results on formaldehyde by two methods.

Analyst.	Sample No. 1, Method I.	Sample No. 2.	
		Method I.	Method II.
	Per cent.	*Per cent.*	*Per cent.*
O. M. Shedd, Lexington, Ky	[a] 36.60	[a] 4.02	4.00
	36.51	4.02	3.98
A. T. Charron, Ottawa, Canada	[a] 37.75	[a] 4.15	.....
	37.55	4.15	.....
		4.04	.....
	[b] 37.53	[b] 4.06	.....
	37.54	4.09	.....
		4.03	.....
H. V. Tartar, Corvallis, Oreg	38.01	4.12	3.91
	38.10	4.12	3.93
L. D. Havenhill, Lawrence, Kans	37.47	4.22	4.18
	37.37	4.32	4.10
W. B. Pope, Washington, D. C	[b] 37.60	[b] 4.22	3.92
	37.60	4.22	3.92
C. C. McDonnell, Washington, D. C	[b] 37.63	[b] 4.10	3.98
	37.65	4.09	3.98
Average	37.49	4.12	3.99

[a] Sample measured and weight calculated from specific gravity.
[b] Portion for determination weighed directly.

O. M. Shedd, in discussing Method II, says:

I used 1 cc of the sample and double the quantities of the solutions directed. The whole was made to 100 cc, and 50 cc used for titration.

A. T. Charron writes as follows:

Direct weighing of the sample was found to be the more satisfactory procedure, as slight variations in temperature materially affect the weight of the solution delivered by the pipette. The figures reported, and many others

obtained in this laboratory, show clearly that more concordant duplicate results can be obtained by weighing the sample directly than by measuring and calculating subsequently the weight from the specific gravity. Owing to the variable quantity of methyl alcohol present in a number of commercial formaldehyde solutions on the market the specific gravity gives no reliable indication of the formaldehyde content of the preparation; moreover, the coefficient of expansion of methyl alcohol being large, the least variation in the temperature of the room notably affects the volume.

The results on formaldehyde sample No. 1, by Method I, are not as close as they should be. It was not considered necessary to direct that correction be made for the acidity of the hydrogen peroxid, but it is thought this may not have been done in all cases. This, as well as the acidity of the formaldehyde solution, must be taken into account and corrections made therefor. The referee's experience has been the same as that noted by Mr. Charron in regard to direct weighing of the formaldehyde, and the method should be changed in this respect. The directions for Method II should be more explicit as to strength of solutions and the amount to be used.

Total Sulphur in Lime-Sulphur Dips.

The Avery method of determining total sulphur in sulphur dips is found in Bulletin 107, Revised, page 34. The cooperative results reported by this method are given in the following table:

Cooperative results on total sulphur in sulphur dips.

Analyst.	Weight of sulphur.	Analyst.	Weight of sulphur.
	Grams per cc.		*Grams per cc.*
O. M. Shedd, Lexington, Ky	0.0451	W. B. Pope, Washington, D. C	0.0457
	.0451		.0457
A. Gordon Spencer, Ottawa, Canada	.0476		.0458
	.0477		.0459
	.0475	C. C. McDonnell, Washington, D. C	.0460
	.0476		.0460
	.0477		.0458
	.0476		.0461
C. E. Bradley, Corvallis, Oreg	.0431	Average	.0459
	.0433		
H. V. Tartar, Corvallis, Oreg	.0443		
	.0446		

This method for total sulphur in sulphur dips is accurate and very satisfactory. The results reported are good. These solutions, particularly if concentrated, deposit sulphur slowly on standing and the differences in results obtained by the different analysts is probably largely due to this cause, some of them having stood longer than others before analysis.

Recommendations.

It is recommended that—

(1) The modification proposed by the referee (Method III) for total arsenic oxid in London purple be given a further trial in comparison with Method I.

(2) Method II, for total arsenious and arsenic oxids in London purple, be dropped from the methods of analysis.

(3) The provisional method for total arsenic oxid in lead arsenate be adopted as official.

(4) Further study be made of methods for the determination of total lead oxid and water-soluble lead and arsenic oxids in lead arsenate.

(5) Method I (precipitation) for soda lye be adopted as official.

(6) The provisional hydrogen peroxid method for the determination of formaldehyde be changed under 2, line 2, to read "3 grams" instead of "3 cc," and adopted accordingly as an official method.

(7) The cyanid method for formaldehyde be changed to read as follows: Under 2, line 8, strike out "dilute formaldehyde solution," and insert the words "formaldehyde solution containing not over 2.5 grams of a 1 per cent solution or the equivalent," and the method as changed be adopted as official.

(8) The provisional hydrogen peroxid method for the determination of total sulphur in sulphur dips be adopted as an official method.

(9) In view of recent work by the writer, in which it has been shown that the presence of chlorids in potassium or sodium cyanids, which are to be used for fumigation work, are very objectionable (on account of the decomposing action of the hydrochloric acid liberated therefrom upon the hydrocyanic-acid gas), it is recommended that a study of Gatehouse's [a] method for the determination of chlorids in cyanids be made, with a view to its adoption as an official method.

REPORT ON WATERS.

By J. K. Haywood, *Referee*, and W. W. Skinner, *Associate Referee*.

It seemed advisable to attempt no comparative work this year, but to spend all the time that could be given to the subject in collecting the best methods for examining the various classes of waters. On account of the magnitude of the work the referee has collected the methods for mineral and sanitary water analysis and assigned the technical and irrigating waters to the associate referee. All of the methods which are offered have been repeatedly tested in the Water Laboratory of the Miscellaneous Division of the Bureau of Chemistry, and it is believed that they represent the most accurate technique of water analysis. The methods are given in detail in Circular 52 of the Bureau of Chemistry, being offered by the referee as provisional, pending an investigation of their accuracy by members of the association.

Following the report on waters, a discussion arose in regard to the necessity of providing a fund to defray the cost of printing the call for the meetings, programmes, and of paying other incidental expenses exclusive of the publication of the Proceedings. The president, in accordance with the vote of the association, appointed a committee, consisting of E. W. Magruder, John Phillips Street, and H. A. Huston, to confer as to the methods of raising a fund for these purposes and to report to the present convention.

[a] Sutton's Volumetric Analysis, 9th ed., p. 201; U. S. Dept. Agr., Bureau of Chemistry, Cir. 10, Rev., p. 6.

REPORT OF COMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By J. K. Haywood, *Chairman.*[a]

(Nitrogen, potash, phosphoric acid, soils, inorganic plant constituents, and water.)

Phosphoric Acid.

It is recommended—

(1) That for paragraph (*a*), page 1, Bulletin 107, Revised, the following be substituted:

(*a*) *Ammonium-citrate solution.*—Dissolve a convenient quantity of commercial citric acid in four times its weight of water; add commercial ammonium hydroxid until a faint odor of ammonia persists when the solution is hot; cool and complete the neutralization as follows: Select four Nessler's jars in which equal columns occupy equal heights, add a carefully purified litmus solution, or preferably a solution of azolitmin, from a burette, taking care that precisely the same volume of indicator is placed in each of the four jars. Fill two of the jars to the 50 cc mark with distilled water neutral to the indicator; add to each of the remaining jars from a burette 2.5 to 5 cc of the citrate solution to be neutralized, and fill to the 50 cc mark. Place the jars containing the indicator and water alone one in front of the other; add to one a drop of strong citric-acid solution and to the other a drop of strong ammonium hydroxid. By looking through both of these jars the neutral tint of the indicator is observed for comparison. Arrange the jars containing the ammonium citrate to be tested in a similar manner, and compare the colors; add citric acid or ammonium hydroxid until the tint produced can not be distinguished from that observed through the pair of jars used for a standard neutral color. It is recommended that the pairs of jars be placed in a box divided into compartments. Narrow slits through each compartment will aid in the comparison of the tints. Bring the neutralized citrate solution to a specific gravity of 1.090, at 20° C. testing by means of a Westphal balance or by a pyknometer.

It was ordered that this method be further studied by the referee, with a view to its consideration for adoption in 1910.

Adopted.

(2) That before the word "flask," in the second line, under "(4) Citrate-Insoluble Phosphoric Acid," page 3, the words "250 cc Erlenmeyer" be inserted.

After some discussion it was ordered that this recommendation be referred to the referee for 1910.

(3) That after the first sentence under the subject head just mentioned, paragraph (4) (*a*), fourth line, the following sentence be added: "The level of the water in the bath should be above that of the citrate solution in the flasks in which the digestion is carried out."

Approved and referred to the association for final action in 1910.

(4) That after the word "contents" in the eleventh line of the same subject head, page 3, the following be substituted: "Through a paper resting on a cone, using suction in the filtration, and washing . . ."

Referred to referee for further trial with view to its adoption.

(5) That for the sentence "Wash thoroughly with water at 65° C." in the twelfth line under the same subject head, page 3, substitute the following: "Wash with water at 65° C. until the volume of the filtrate is about 350 cc, allowing time for thorough draining before adding new portions of water."

This recommendation was referred to the referee for further study and action in 1910.

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[a] In the absence of the chairman this report was presented by J. P. Street, Connecticut.

(6) That the association appoint a special committee to confer with experiment stations with the view of securing their cooperation in instituting culture experiments for determining the relative availability of the phosphoric acid in basic slags, this committee to make a report annually on the work undertaken and to present a complete summary of the results and also to make appropriate recommendations concerning laboratory methods of examining basic slags after a period of five years.

Adopted.

(7) That the Wagner method for estimating the availability of phosphoric acid in slags be brought up for provisional adoption at the next meeting.

Adopted.

NITROGEN.

It is recommended—

(1) That in Bulletin 107, Revised, page 8, line 4, after the word "time," the following be inserted: "Allow the flask to stand without heat for not less than six hours, or for a shorter time with shaking at regular intervals,[a] the intention being to secure complete solution of the nitrate."

Lost.

(2) That in Bulletin 107, Revised, page 8, under "(3) Determination," line 5, after the word "and," the following be inserted: "allow the flask to stand without heat for not less than six hours or for a shorter time with shaking at regular intervals,[a] the intention being to secure complete solution of the nitrate."

Lost.

POTASH.

It is recommended—

(1) That Drushel's modification of the cobalti-nitrite method for the determination of potash be given a further trial during the ensuing year.

Adopted.

SOILS.

It is recommended—

(1) That the modified J. L. Smith method for total potassium be made an official method of this association, and that the clauses "and transfer to a filter" and "after washing free of chlorids" in this method be replaced by the following sentence: "After washing four or five times by decantation with hot water, throw on the filter and wash well, 250 to 300 cc of wash water being sufficient."

Approved and referred to the association for final action in 1910.

(2) That the magnesium-nitrate method for total phosphorus be made an official method of this association.

Approved and referred to the association for final action in 1910.

(3) That the sodium-peroxid-fusion method for total phosphorus be made an official method.

Approved and referred to the association for final action in 1910.

(4) That the Drushel modification of the cobalti-nitrite method, in connection with the J. L. Smith fusion method, be tested by the association the coming year as a method for total potassium.

Adopted.

WATER.

It is recommended—

(1) That the methods for mineral, sanitary, technical, and irrigation water analysis collected and tested by the referee and associate referee this year be

[a] When the nitrate nitrogen present exceeds 1.50 per cent, the six-hour method is recommended.

printed for criticism of the members, with a view to their adoption next year as provisional methods of the association. (See Circular 52, page 4.)

Adopted.

INSECTICIDES.

It is recommended—

(1) That the modification proposed by the referee (Method III) for total arsenic oxid in London purple be given a further trial in comparison with Method I.

Adopted.

(2) That Method II, for total arsenious and arsenic oxids in London purple be dropped from the methods of analysis.

Adopted.

(3) That the provisional method for total arsenic oxid in lead arsenate be adopted as official.

Approved and referred to the association for final action in 1910.

(4) That further study be made of methods for the determination of total lead oxid and water-soluble lead and arsenic oxids in lead arsenate.

Adopted.

(5) That Method I (precipitation) for soda lye be adopted as official.

Adopted.

(6) That the provisional hydrogen-peroxid method for the determination of formaldehyde be changed, under 2, line 2, to read "3 grams" instead of "3 cc," and adopted accordingly as an official method.

Adopted.

(7) That the provisional cyanid method for formaldehyde be changed to read as follows: Under 2, line 8, page 33, Bulletin 107, Revised, strike out "dilute formaldehyde solution" and insert the words "formaldehyde solution containing not over 2.5 grams of a 1 per cent solution or the equivalent," and the method as changed be adopted as official.

Approved and referred to the association for final action in 1910.

(8) That the provisional hydrogen-peroxid method for the determination of total sulphur in sulphur dips be adopted as an official method.

Adopted.

(9) That a study be made of Gatehouse's method for the determination of chlorids in cyanids, with a view to adoption as an official method.

Adopted.

REPORT OF COMMITTEE ON THE TESTING OF CHEMICAL REAGENTS.

By L. F. KEBLER.

During the past year a number of manufacturers of chemical reagents were interviewed in person and by correspondence relative to a satisfactory nomenclature for chemical reagents. There appeared to be a general feeling of dissatisfaction, not only among the chemists, but also among manufacturers of chemical reagents, with the nomenclature at present in vogue. All were desirous of securing or adopting a nomenclature which would definitely and specifically state what was meant when a certain commodity was called for under a certain designation. With few exceptions no one volunteered to make any suggestions, and furthermore many were of the opinion that it would be difficult to devise a satisfactory nomenclature for these products. The committee

therefore can report progress only, and it is recommended that the committee be instructed to continue the subject of devising a suitable nomenclature for chemical reagents.

At the close of the presentation of this report it was moved and carried that the report of the committee be accepted and the committee continued.

The committee on the revision of methods made no general report other than that made by the respective subchairmen of Committees A, B, and C. The reports of these subcommittees were approved by the committee as a whole subsequent to the meeting, many of its members having been absent.

SECOND DAY.

FRIDAY—MORNING SESSION.

REPORT ON FOOD ADULTERATION.

By H. E. Barnard, *Referee.*

In the control of food adulteration by official laboratories during the past year the more common forms of adulteration have for the most part needed less attention than formerly, because of the satisfactory influence of good food laws and an increasingly vigorous enforcement. The work of the official chemist tends, therefore, rather toward the study of specific instances, which, because of their importance both to the consumer and to the honest manufacturer, need more through investigation. Thus, while spice adulteration is less common than before the passage of food legislation, while less cottonseed oil reaches the market bearing the brand of Italian or French oils than a few years ago, while preservatives are not now commonly found in milk, while canned goods seldom contain bleaches and artificial sweeteners, while the character of maple products has wonderfully improved, yet there still remains a broad field which requires investigation. Such questions as the deterioration of products in cold storage and the use of chemical preservatives, of bleaches, and of coloring agents are still mooted, and must continue to be so until more work has been done in those lines.

One of the most interesting problems which have received attention during the year just past has been that of the bloated oyster. It has been apparent to food officials in the Central States, and to a less extent in the Coast States, that the oyster as it reached its market was very different in character from the oyster opened and consumed directly from the shell. And that fact, coupled with the peculiar trade condition which allowed oysters to be purchased in Baltimore, shipped halfway across the continent, and then sold at a price below what they cost on the wharves of Chesapeake Bay, led chemists to attempt a solution of the problem. The answer is, of course, obvious. The retailer sells some oysters and a great deal of melted ice—oyster juice the consumer is told. The absence of any standard of water content for shucked oysters and the fact that the common practice of washing the freshly shucked oysters allows the admixture of indefinite amounts of water in a manner considered entirely justifiable have made it very difficult to determine at the laboratory the exact character of the goods. The action of a majority of the Central States in specifically prohibiting the sale of oysters shipped in contact with ice or in ice water makes it important that accurate and reliable data on the normal water content of oysters shall be soon available.[a]

The high cost of cider vinegar and the low cost of imitations explain the prevalence of the adulteration of this product. The analytical methods of a

[a] Since this report was written Food Inspection Decision 110 has been issued by the U. S. Department of Agriculture, declaring it to be "unlawful to ship or to sell in interstate commerce shucked oysters to which water has been added either directly or in the form of melted ice."

few years ago which were considered quite sufficient to detect adulteration are now inadequate, though from time to time methods for determining lead numbers, alkalinity of ash, polarization, etc., have been added. The improvement of methods and the development of new tests which will enable us to detect with certainty mixtures of the new vinegars made from raw sugar, acetic acid, apple pomace, fruit ethers, invert sugars, phosphates, etc., are most necessary.

The methods for determining whether or not a given sample of butter is renovated or a mixture of renovated and genuine should be further studied, and the adulteration of honey by feeding bees sugars or by mixing invert sugars in the extracted honey presents another problem for the solution of the food chemist.

Present methods of beer analysis fail to determine the true character of the mash from which the beer was brewed, and the adoption of standards for beers by many of the States makes further research into the chemical factors of all malt and malt substitute beers very necessary. Some work has been done along this line this past year which will be reported in a supplementary paper (see page 87).

How can the chemist best determine whether durum wheat flour has been mixed with winter wheat flour; whether the nitrites present are due to the bleacher or imparted to the flour by an adjacent bag of heavily bleached flour; whether the salts in a mineral water are naturally present or added with a scoop out of a barrel; whether milk has been pasteurized or only heated; whether fat has been abstracted from milk in violation of that section of our laws which prohibits the removal of any valuable constituent, especially if the original milk is rich and the skimmed milk still standard as to its butter fat content; whether eggs are really strictly fresh or taken from careful storage; whether the color added to butter is put there by the farmer to make his product uniform or to turn December into June in palpable violation of the principles underlying food legislation? The fact that nearly every one of these questions is now under discussion and that apparently satisfactory methods have recently been worked out in a number of different cases leads to the belief that all may be solved with careful study and cooperative work.

The very satisfactory progress made by the associate referees and reported in the Proceedings of the Twenty-fifth Annual Convention of the Association has been duplicated by the work done this present year, although the early date of the meeting has made it impossible to secure full reports from collaborators. Reports have been made which will later be discussed more fully by the associate referees on seventeen subjects, all being covered with the exception of saccharine and cereal products.

In concluding this my second report as referee on food adulteration, I wish to thank the chemists who have by their willing cooperation and painstaking work contributed so much to the important work of improving methods for the analysis of foods.

REPORT ON COLORS.

By H. M. Loomis, *Associate Referee.*

Plan of Work.

The work on colors by the referee of this year consisted in the preparation and distribution of ten samples of three general classes of food products to which coloring agents had been added; in the correlation of the data obtained

from the cooperating chemists, Messrs. F. O. Woodruff, Hare, and Bjerregaard; in a personal investigation of the problems presented by the samples; and in a continuation of the work of last year in the testing of the purity of the colors used in the preparation of Circular 35, Bureau of Chemistry, on "The solubility and extraction of colors, etc."

The colored samples sent out comprised four pastries, three fats, and three vanilla extracts, the cooperators being requested to do as much work as possible on the identification of the colors used therein. The samples were as follows:

PASTRIES.

I. Flour pastry colored with saffron.
II. Flour pastry colored with 0.006 per cent mixture of three parts Naphthol Yellow S and one part Orange I.
III. Flour pastry with 10 per cent egg yolk.
IV. Flour pastry 94 per cent, with lard 6 per cent and 0.004 per cent butter yellow (S. & J. 16).

FATS.

I. Lard and approximately 0.04 per cent carrotin.
II. Lard and butter yellow (S. & J. 16).
III. Lard and 5 per cent palm oil.

VANILLA EXTRACTS.

I. Fifty per cent extract of vanilla bean (No. III) and 50 per cent imitation extract prepared by digesting prunes with 40 per cent alcohol and adding 0.25 per cent vanillin.
II. Extract of vanilla bean (No. III) and 50 per cent by volume imitation extract prepared by diluting caramel solution to same depth of color as true extract and adding 0.25 per cent vanillin.
III. Extract of vanilla bean with 50 per cent alcohol.

The reports of the collaborators, although showing that much time and care had been expended, chiefly indicated the difficulties which exist in identifying colors in certain food products, and particularly such colors as are of vegetable origin. The results being of a tentative nature, only an abstract is given.

Vanilla Extracts.

The work of the referee has shown that Jagerschmid's test for caramel in beer and wine [a] gave a reaction for caramel with all three extracts, and is evidently of no value in detecting caramel in these products. The phenylhydrazin hydrochlorid method for caramel [b] was tried with the extracts, except that 2 cc each of zinc chlorid and caustic potash solutions were used, or more potash solution if necessary to produce copious precipitates, and, after almost neutralizing the excess of acetic acid, the solution was filtered. After boiling one-half hour extract No. 1 gave a brown turbidity and slight precipitate, and two samples of genuine extract gave no precipitate and were nearly clear. No. 2 gave a marked brown precipitate. After standing about five hours, extract No. 2 gave a decided brown precipitate, while extracts Nos. 1 and 3 gave none. The fuller's earth method of extracting caramel [b] failed to give any results of value.

The dealcoholized extracts were treated in acid and neutral solution with immiscible solvents, ethyl acetate, amyl alcohol, and acetone after saturating with salt, but failed to give distinctive reactions except in the case of acetone from acid solution saturated with salt, which process both the referee and Mr. Woodruff found was of assistance in the separation of true vanilla color from

[a] Zts. Nahr. Genussm., 1909, 17: 269.

[b] U. S. Dept. Agr., Bureau of Chemistry, Bul. 65, p. 71.

caramel color. Tolman's quantitative modification of Marsh's color test gave the following results on the per cent of color in the amyl layer:

	Per cent.
Extract No. 1	55
No. 2	50
No. 3	90

DETERMINATION OF RESINS.

Fifty cc of the extracts were dealcoholized by twice evaporating to 25 cc; they were then acidified and filtered. The filter was washed thoroughly with cold water, dried, and extracted with 95 per cent alcohol, till all soluble matter was extracted. The alcoholic solution was evaporated to dryness, dried, and weighed as resins. The following results were obtained:

	Resins (grams per 100 cc).
Extract No. 1	0.034
No. 2	.027
No. 3	.071

PASTRIES.

The first difficulty presenting itself was the isolation of the dye in such a form that it might be subjected to the various tests with reagents and on wool. Nos. 1 and 2 were best extracted with 70 per cent alcohol and Nos. 3 and 4 with either sulphuric or petroleum ether. The work of referee and of collaborators consisted mainly in making the usual tests with hydrochloric acid, sulphuric acid, caustic alkalies, ammonium hydroxid, stannous chlorid, etc., on the dry pastry, or on a solution of the extracted dye.

FATS.

As in the case of the pastries the principal difficulty lay in the separation of the dye from the fat, since those solvents which extracted the color also dissolved the fat. By acidifying slightly and stirring with thoroughly chilled 70 per cent alcohol, filtering, repeating this several times, evaporating, and diluting with water, Mr. Woodruff was able to identify carrotin in fat No. 1 from the fiber and solubility reactions. With fat No. 2 he also was able to separate and identify the dye by dissolving in petroleum ether, washing thoroughly, adding tenth-normal potassium hydroxid, shaking, and allowing to stand, after which the separated color solution was drawn off and subjected to the usual tests. The referee and Messrs. Hare and Bjerregaard made tests of the fastness to light and air of the colored fats. It was found that the color of palm oil bleached very easily in the presence of air and diffused daylight, the fat being nearly decolorized in eighteen hours. Carrotin is somewhat less fugitive and butter yellow is quite fast. All three colors were quite fast when exposed to light but out of contact with air. The referee also tried Moore's test,[a] and the Crampton and Simond's test,[b] but neither test answered its purpose in detecting carrotin or palm oil, respectively. A more extended study should be made of these methods, however, before they are adjudged useless for this purpose.

The results of the entire investigation indicate that it is generally of little use to apply color tests unless the color can be isolated in a state of reasonable purity, and work along this line is urgently needed, particularly in the field of

[a] Leach, Food Inspection, 1907, p. 435.

[b] J. Amer. Chem. Soc., 1905, 27: 270.

vegetable colors. In the testing of colors used in the preparation of Circular 35, the referee has been able to try those given on pages 4, 5, and 6 of that circular, and all of them were found to be unmixed colors with the exception of eosin (Grübler), which appeared to contain two colors of that class of dyes. The methods used in this work are described in last year's report to the association.[a] The recommendations of 1908 are again called to the attention of the association for the coming year.

SUGGESTED MODIFICATION OF THE WINTON LEAD NUMBER.

By S. H. Ross.

The suggested modification of the Winton lead number, especially as applied to mixtures of maple and cane sugar sirups, and details of the work on known samples on which the modification is based, are to be found in Circular 53 of the Bureau of Chemistry. The modified method may be briefly stated as follows:

Transfer 25 grams of the sirup to a 100 cc flask, using about 25 cc of distilled water,[b] add 10 cc of potassium sulphate solution (7 grams per liter); then 25 cc of lead subacetate solution of the strength specified by Winton.[c] Make up to the mark, shake thoroughly, and allow to stand three hours. Filter, rejecting the first portion of the filtrate. Determine the lead sulphate from 10 cc of the clear filtrate as usual. Run a blank, using 25 grams of a pure cane sugar sirup instead of the sirup to be tested. From the difference in the weight of the lead sulphate obtained calculate the lead number as follows: Subtract the weight of the lead sulphate, obtained from 10 cc of the clear filtrate, from that obtained from 10 cc of the cane sugar sirup blank filtrate. The remainder, expressed in grams, multiplied by 27,325[d] equals the modified lead number or percentage of lead precipitated by the sirup.

The consideration of this method by the associate referee on saccharine products for adoption as a provisional method is suggested.

VARNISHES ON CHOCOLATE AND CONFECTIONERY.

By B. H. Smith.

Nature of Chocolate Varnishes.

Many of the chocolate manufacturers of the United States are using a shellac or other form of spirit varnish on their plain, or "bitter," chocolate when put up for the retail trade, and a considerable amount of confectionery, especially penny chocolate goods, peanut bars, etc., is finished with a coating on one or more sides. Zipperer refers to the practice of coating chocolate in his book, "The Manufacture of Chocolate," and states that "Peruvian balsam is very much used as a perfume." He also says that "Benzoin is almost exclusively used for the preparation of chocolate varnish and confiture lac, which are prepared by dissolving 25 to 45 grams of the lac body in 100 grams of strong spirits. The lac body may contain varying quantities of benzoin and bleached

[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. 122, p. 38.

[b] Freshly boiled distilled water should be used throughout.

[c] J. Amer. Chem. Soc., 1906, 28: 1205.

[d] This factor is obtained by dividing the lead factor (0.68312) by the number of grams of sirup in the 10 cc used (2.5 grams) and multiplying by 100.

shellac. The decorations of chocolate are painted with this lac in order to give a glistening appearance and greater durability." Merck's Index, under the uses of Siam benzoin, gives "chocolate coating," and under Peruvian balsam "chocolate manufacture." As the majority of food laws refer to the "coating" of food products, the identification and, when possible, the quantitative determination of such varnishes upon food products becomes of interest to food chemists.

As these varnishes are applied in alcoholic solution (either by means of a brush or by dipping), it follows that alcohol is an excellent solvent for their removal. The oxidation to which the film of varnish has been subjected, however, tends to render resins less soluble in the ordinary solvents; but 90 per cent alcohol, particularly when warm, has served to remove without great difficulty all varnishes that the writer found in actual use upon chocolate or confectionery. The residue from the alcoholic solution may contain small amounts of fatty acids, fats, sugar, coloring matter, etc., and the method to be employed in eliminating these compounds must necessarily depend on the nature of the varnish. Preliminary work of a qualitative nature is therefore essential at the beginning of the examination.

Shellac.

Shellac is the coating most extensively used, and its identification and closely approximate determination are easily accomplished. The grade usually employed is the ordinary shellac of commerce, which is very often adulterated with rosin. The alcoholic solution of shellac gives a bright violet color upon the addition of alkali, and lead acetate gives a violet precipitate. Shellac is soluble in amyl and methyl alcohols as well as ethyl alcohol and in glacial acetic acid. It is not soluble in petroleum ether, except the wax present (usually 3 to 5 per cent), and this insolubility may be made use of in its purification. To accomplish this the alcoholic residue may be evaporated to dryness on the water bath with a liberal quantity of clean sand, stirring with a rod toward the end of the evaporation. The sand mixture is then repeatedly washed with petroleum ether to remove traces of fat, fatty acids, etc., and subsequently with hot water to remove the small amounts of sugar, cocoa red, or other extractive matters which may be present.

The general method of purifying shellac suggested by Langmuir may be applied to coatings of shellac removed from food products. The residue from the alcoholic extract is dissolved in warm dilute sodium carbonate, cooled, and filtered to remove wax and any other insoluble matter, and the shellac is then precipitated from the filtrate by the addition of an acid. Allow the finely divided precipitate to stand until it has settled out and then filter through a Gooch or Buchner filter, washing as before with petroleum ether and hot water.

Upon the purified resin the iodin, acid, and ester numbers should be determined. The iodin number is of especial value, as practically all of the spirit soluble resins give much higher results than shellac, which, when unadulterated, should not give an iodin number exceeding 20. For this determination 2 grams or more of the sample should be used, glacial acetic acid being employed as the solvent. If the flask is gently warmed the shellac dissolves quite readily. The Hanus method gives excellent results.

The Balsams.

Peruvian balsam and gum benzoin are indicated by the fragrance of the alcoholic solution of the coating, particularly when warmed. The saponification, acid, and ester numbers, determined as outlined by Dieterick, aid in their identification and differentiation. If a pound or more of the chocolate or con-

fectionery is available, benzoic acid may be sublimed from the alcoholic extract and identified by the usual tests. Cinnamic acid and vanillin may also be tested for in the sublimate. Both Siam and Sumatra gum benzoin give a bright red color with concentrated sulphuric acid.

Rosin in Coatings.

Because of its cheapness and ready adaptibility to the purpose, rosin is used very extensively as an adulterant of shellac, of the balsams, and of all spirit varnishes, and should be tested for in all samples of food coatings of this nature. Its high iodin and acid numbers and low ester number are characteristic when a large amount is present, and these determinations are of value in interpreting results when it is used in smaller quantities. Several qualitative tests for rosin are satisfactory, the following having given very good results in our hands:

The Lieberman-Storch-Morawski reaction is easy of execution and when applied to the residue of the alcoholic extract of a material which contains rosin it is very delicate. A small quantity of the residue from the alcoholic extract is dissolved in acetic anhydrid by gentle warming in a small porcelain crucible. After cooling, a drop or two of sulphuric acid of 1.53 specific gravity is added, when a violet red coloration is immediately produced.

Another good color test is that described by G. Halpen,[a] and depends upon the fact that rosin gives a violet and blue coloration with a solution containing bromin and phenol; water or alcohol interferes with the reaction. A dry portion of the residue from the alcoholic extract is mixed with 1 or 2 cc of a solution of crystallized phenol in carbon tetrachlorid (1:2) in a porcelain crucible, and bromin vapor from a flask containing bromin dissolved in carbon tetrachlorid is allowed to fall into the crucible.

With both of these tests blanks should be run on an alcoholic extract of a sample of the material under examination which does not contain any of the coating.

REPORT ON FRUIT AND FRUIT PRODUCTS.

By H. C. Gore.[b]

Attention has been called to the study of methods for the determination of total acid by two articles which have recently appeared in the Proceedings. Street[c] gives the general status of the question when he says, in discussing the use of indicators in the titration of the acids in cattle feeds, "The question to be settled first of all is just what do indicators indicate." Hortvet,[d] as a result of studies on the determination of acid in wines, strongly recommends phenolphthalein as the indicator to be employed in such cases. Fruit juices appear to be exceptionally favorable material for a study to determine the best indicator for use in the estimation of total acid. Phases of the work studied this year are as follows:

(1) Titration of grape juices, using litmus in three slightly different ways, and phenolphalein.

(2) Titration of 15 fruit juices, using litmus solution and phenolphthalein and preparing the samples for titration by different methods.

(3) The influence of carbon dioxid and methods for its elimination.

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[a] Analyst 1903, 28:9.

[b] At the request of the referee, C. B. Cochran, Mr. Gore conducted the cooperative work on this subject.

[c] U. S. Dept. Agr., Bureau of Chemistry, Bul. 122, p. 161.

[d] Ibid., p. 23.

(1) Titration of Grape Juices Using Litmus in Three Ways and Phenolphthalein.

In this study cooperation was secured, the following circular letter being sent with samples to a list of collaborators:

May 19, 1909.

* * * Two samples of sterilized grape juice in mailing tubes accompany this letter. Sample No. 1 is a cold-pressed juice and contains about 0.056 per cent of tannin and coloring matter. No. 2 is a hot-pressed juice and contains about 0.402 per cent of tannin and color. More of each sample will be sent you if required. A small piece of Merck's litmus, which is nearly or entirely free from foreign coloring matter, will be found in one of the tubes.

Four methods are proposed for cooperative work, as follows:

(1) The provisional method of the association as applied to wines, Bulletin 107, Revised, page 86, but using 10 cc samples and neutral litmus paper and titrating until no red tinge is imparted to the paper by a drop of the mixture.

(2) The same method, but titrating until a drop of the mixture produces no reddish tinge when added to small drops of a dilute litmus solution on a white porcelain or ground-glass surface. In preparing the solid litmus for use, rub up with water and add dilute hydrochloric acid until the mixture is of a slight permanent purple tinge.

(3) The same method, but titrating until a blue tinge is imparted to litmus paper by a drop of the mixture.

(4) The provisional method of the association, as applied to fruits and fruit products, Bulletin 107, Revised, page 79, using phenolphthalein as indicator.

In Methods 1, 2, and 3 the following procedure should be followed for preparing a sample for titration: Run 10 cc into about 100 cc of boiling water contained in a beaker of from 150 to 250 cc capacity. Warm to insipient boiling and titrate at once, using tenth-normal soda free from carbonates. In Method 4, follow the same procedure, but use a larger beaker and dilute as far as appears to be necessary, in order to see the phenolphthalein color. The first three methods of recognizing the end point can be employed on the same 10 cc portions. * * * Express results as cubic centimeters of tenth-normal alkali required for 10 cc of juice.

The results obtained and the opinions of the analysts are as follows:

Table 1.—*Cooperative results on acid determination by four different methods.*

SAMPLE NO. 1. COLD-PRESSED CONCORD GRAPE JUICE.

Analyst.	Cubic centimeters of tenth-normal alkali required for 10 cc of juice.				Per cent of acid indicated.[a]			
	Method 1.	Method 2.	Method 3.	Method 4.	Method 1.	Method 2.	Method 3.	Method 4.
Azor Thurston	10.50	10.00	10.80	12.00	105.0	100	108.0	120.0
A. M. Henry	10.30	10.40	10.40	10.55	99.0	100	100.0	101.0
E. C. Hill	9.50	10.00	10.40	12.30	95.0	100	104.0	123.0
A. L. Davison	9.75	9.75	10.00	11.65	100.0	100	103.0	119.0
W. B. Kelling	8.70	8.80	8.85	10.20	99.0	100	101.0	116.0
J. R. Eoff	10.20	10.28	10.50	12.25	99.0	100	102.0	119.0
H. C. Gore	9.60	10.25	10.72	12.37	94.0	100	105.0	121.0
Average[b]					98.6	100	103.8	119.6

SAMPLE NO. 2. HOT-PRESSED CONCORD GRAPE JUICE.

Azor Thurston	12.50	11.60	12.80	14.40	108	100	111.0	124
A. M. Henry	9.25	9.40	9.40	.....	98	100	100.0	.....
E. C. Hill	10.10	11.40	11.20	13.90	95	100	98.0	122
A. L. Davison	10.25	10.85	11.40	13.65	94	100	105.0	126
W. B. Kelling	11.15	11.30	11.35	12.70	99	100	101.0	112
J. R. Eoff	11.63	11.85	11.85	14.85	98	100	100.0	125
H. C. Gore	10.95	11.70	12.20	13.70	94	100	104.0	117
Average[b]					98	100	103.3	121

[a] Based on results of Method 2 as 100 per cent.
[b] Omitting the figures of Mr. Henry.

COMMENTS OF ANALYSTS.

Azor Thurston: With Method 4, sample No. 2, the reading was quite unsatisfactory, even when diluted to 500 cc.

A. M. Henry: Method 1 is very unsatisfactory, as it is hard to distinguish the end point. By Method 4 the results were too high, being especially unsatisfactory for sample No. 2. I would prefer to use both Methods 2 and 3 as a check on each other.

E. C. Hill: It seems to me that Method 2 is best for these colored juices.

A. L. Davison: The end reaction using litmus paper was difficult to determine. The color of both samples interfered with the use of phenolphthalein as indicator.

W. B. Kelling: No. 2 probably most accurately indicates the neutral point. Method 4 indicates the end reaction very indefinitely.

J. R. Eoff: End points, using litmus, very satisfactory. Using phenolphthalein, could get no end point when determinations were carried on in a beaker. End point with sample No. 1 was satisfactory when a large volume of boiled, cooled, and neutralized water was used and the titration conducted in a porcelain evaporating dish. Results were less satisfactory with sample No. 2.

H. C. Gore: Method 1, solution still acid, as the litmus solution (made as in Method 2) was reddened. Method 2, end point sharp. Method 3, end point fairly sharp, but difficult to recognize. Method 4, end point difficult to recognize. In case of sample No. 2 the end point by Method 4 was very hard to determine.

The results obtained by the collaborators agree fairly well when the figures obtained by Method 2 are considered as representing 100 per cent and the other figures are given corresponding values. The differences between the amounts of acid indicated by litmus and by phenolphthalein are found to be about 20 per cent. This variation is greater than would be caused by the failure of litmus to indicate all of the organic acid present, and should be the subject of special study.

(2) Titration of Fruit Juices Using Litmus Solution and Phenolphthalein, and Preparing the Samples Differently.

The juices were prepared by the writer and titrated by A. L. Davison under his directions. The samples were free from appreciable amounts of sediment, and with the exception of two samples of fresh peach juice, had all been sterilized by heating in glass to temperatures not exceeding 75° C. Recently boiled distilled water was used in all of the dilutions. Ten cubic centimeter portions of the juices were neutralized with tenth-normal soda, which contained a small proportion of barium hydrate. Litmus free from other coloring matters was used, and the phenolphthalein employed was neutralized.

In this study litmus and phenolphthalein were first compared, employing the procedure used in the cooperative work, Methods 2 and 4, i. e., 10 cc samples were added to 100 cc of boiling water; the mixture was warmed to incipient boiling, and titrated with tenth-normal soda, using litmus and phenolphthalein successively. In using the litmus solution, the end point was considered to be reached when a drop of the mixture produced no red tinge when mixed with a drop of sensitive blue litmus solution on a white porcelain surface. The phenolphthalein was used in the solution. A blank was not run, i. e., a solution to which no indicator was added,[a] and hence the values found when using phenolphthalein in the case of the highly colored juices are probably somewhat high, while the results on the slightly colored juices, such as peach, apple,

[a] J. Ind. Eng. Chem., 1909, 1 : 436.

strawberry, pineapple, and orange, are probably correct. The figures are given in the first half of Table 2.

In five cases it was necessary to dilute to 500 cc in order to detect the end point due to phenolphthalein; in three other cases it was very difficult to see the end point with phenolphthalein even after dilution, and when using black raspberry juice it was impossible. The average differences between the amounts of acid shown amount to 8.7 per cent, with a maximum of 28 per cent and a minimum of 1.8 per cent. These differences can hardly be due to the failure of litmus to titrate the organic acids. Results obtained by the writer [a] using litmus in the manner just described, demonstrated that from 96 to 99 per cent of organic acids shown by phenolphthalein are indicated by litmus. The cause of these differences should, therefore, be given further study.

Part II of Table 2 gives results obtained by slightly varying the procedure for titrating when using litmus. These should be compared with those shown in the first column of Part I. The first change tried consisted in omitting the heating of the diluted sample. The average result of 26 titrations shows practically no change in the amount of alkali, 15.47 cc being required, whereas 15.50 was the average figure when the solutions were heated to boiling. The next change consisted in omitting both the heating and diluting and passing a rapid current of air through the samples for one minute to remove carbon dioxid, if present. Here again practically no change was produced in the average. In the third set of titrations the dilution and heating were omitted, but the samples were not aerated, and the average results were again practically identical with the others. In the case of the darker juices, which retained their color on neutralizing, dilution was found desirable, as the amount of color in the drop transferred to the test plate was apt to obscure the red tinge given to the litmus solution just before the end point was reached.

From these experiments it may be concluded that, except in case of deeply colored juices, which retain their color on neutralizing or which contain carbon dioxid, the dilution and heating to boiling before titrating may be omitted.

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[a] J. Ind. Eng. Chem., 1909, 1: 436.

TABLE 2.—*Results of titrating fruit juices in various ways.*

[Results expressed in terms of alkali required to neutralize 100 cc of sample.]

Description of juice.	I. Comparison of litmus and phenolphthalein.				II. Litmus used and procedure varied in three ways.		
	Litmus.	Phenolphthalein.	Difference.	Per cent difference.[a]	Diluted but not heated.	Undiluted, unheated, aerated.	Undiluted, unheated, not aerated.
Peach, freshly pressed	[b] 11.8	12.7	0.90	7.6	[b] 11.65	.....	.....
	[b] 11.7	12.45	.75	6.4	[b] 11.70	.....	.....
Do	11.4	12.55	1.15	10.1	12.10	11.4	11.9
	11.35	12.65	1.30	11.5	12.10	11.1	12.1
Apple (R. I. Greening), sterilized	[b] 5.45	6.25	.80	14.7	[b] 5.67	.....	.....
	[b] 5.50	6.25	.75	13.6	[b] 5.50	.....	.....
Apple (Jonathan), sterilized	7.20	7.70	.50	6.9	7.45	7.35	7.45
	7.10	7.80	.70	9.9	7.55	7.40	7.44
Grape (Catawba), sterilized	11.92	13.12	1.20	10.1	12.00	12.05	12.10
	11.98	13.18	1.20	10.0	11.85	12.15	12.00
Strawberry (Lady Thompson), sterilized	18.90	19.25	.35	1.8	18.95	19.00	18.90
	18.95	19.35	.40	2.1	18.95	19.00	19.00
Huckleberry, hot pressed, sterilized	7.55	[c] 9.55	2.05	27.2	7.30	[d] 7.35	[d] 7.30
	7.50	[c] 9.60	2.10	28.0	7.30	[d] 7.40	[d] 7.50
Huckleberry, cold pressed, sterilized	6.90	[c] 7.45	.55	10.8	6.65	6.90	6.83
	6.90	[c] 7.55	.65	9.4	6.72	6.95	6.80
Pineapple (Red Spanish), sterilized	13.68	14.60	.92	6.7	13.60	13.70	13.60
	13.70	14.80	1.10	8.0	13.60	13.60	13.60
Red currant (Fay), sterilized	36.85	37.60	.75	2.0	36.50	36.85	36.70
	36.80	37.40	.60	1.6	36.60	36.95	36.85
Blackberry, wild, sterilized	16.15	[c] 16.50	.35	2.2	16.05	16.45	[e] 16.10
	16.25	[c] 16.50	.25	1.5	16.15	16.20	[e] 16.45
Blackberry (Early Harvest), sterilized	12.4	[c] 14.00	1.60	12.9	12.40	12.60	[e] 12.60
	12.5	[c] 13.75	1.25	10.0	12.55	12.60	[e] 12.80
Blackberry, wild, sterilized	18.65	[c] 19.55	.90	4.8	18.60	18.65	[e] 18.65
	18.60	19.40	.80	4.3	18.50	18.70	[e] 18.50
Red raspberry, sterilized	24.5	26.00	1.50	6.1	24.10	24.65	24.80
	24.6	26.10	1.50	6.1	24.12	24.65	24.60
Black raspberry, sterilized	[b] 18.85	(f)	.....	.....	[b] 18.75	(d)	(d)
	[b] 18.75	(f)	.....	.....	[b] 18.75	(d)	(d)
Orange (Washington Navel), sterilized	15.5	16.55	1.00	6.5	15.30	15.20	15.40
	15.2	16.50	1.30	8.5	15.25	15.45	15.45
Average	15.50	.....	.....	8.7	15.47	15.55	15.59

[a] Figures based on litmus results considered as 100 per cent.
[b] Omitted from averages.
[c] Diluted with 500 cc of water.
[d] Litmus unsatisfactory on account of the intense color of the juice near the neutral point.
[e] Phenolphthalein very difficult to use.
[f] Impossible to use phenolphthalein.

(3) Influence of Carbon Dioxid and Methods for its Elimination.

It is well recognized that the presence of carbon dioxid in fruit juices, wines, and vinegars causes an excess of the standard alkali to be used in titrating, and high results are obtained. The error produced by the carbon dioxid is variable on account of its volatility, the amount actually fixed by the alkali in a given titration depending on minor points in the manipulation, such as rate of stirring, etc. The methods usually direct heating before titrating. Some writers [a] even recommend heating under a return condenser in case the sample contains volatile acid. These facts led the writer to determine how readily carbon dioxid could be removed from solution by simple washing with a current of air, and what effect such aeration would have on the volatile acid content.

Ordinary distilled water was found to contain appreciable amounts of carbon dioxid, which were, however, readily removed by washing with common air.

[a] Hortvet, J. Ind. Eng. Chem., 1909, 1: 31.

In one experiment about 5 gallons of distilled water were treated with a rapid stream of air, and the following table shows that the carbon dioxid present was rapidly eliminated by aerating. Results are expressed as cubic centimeters of hundredth-normal sodium hydroxid required to produce a permanent pink when added to 100 cc of water, phenolphthalein being the indicator.

TABLE 3.—*Results of aerating distilled water for varying periods.*

Time of aerating.	N/100 alkali.
Minutes.	*cc.*
0	0.75
7	.35
16	.20
27	.20
50	.15
60	.20

One hundred cubic centimeters of this water, after boiling and cooling, required 0.1 cc of hundredth-normal sodium hydroxid.

Experiments were now made to determine how rapidly carbon dioxid may be driven out of carbonated water by washing with air. A sample of charged water was prepared by treating aerated distilled water with carbon dioxid. This solution required 5 cc of decinormal baryta for each 10 cc, determined by running the sample into excess of the baryta and titrating with decinormal oxalic acid, using phenolphthalein as indicator. Ten cubic centimeters also required 24.8 cc of hundredth-normal sodium hydroxid when suitable precautions were taken during the titration to prevent the loss of carbon dioxid by volatilization. The following table shows the results obtained on aerating 10 cc portions of this carbonated water, using hundredth-normal alkali in titrating, and phenolphthalein as indicator. Practically all of the carbon dioxid was removed by treating for one minute with 2 liters of air.

TABLE 4.—*Results of aerating carbonated water for varying periods.*

Volume of air used.	Time.	N/100 sodium hydroxid.
cc.	*Minutes.*	*cc.*
500	0.5	1.2
500	.5	1.2
1,000	.5	.6
1,000	.5	.6
2,000	.5	.2
2,000	.5	.2
2,000	1.0	.1
2,000	1.0	.1
4,000	1.0	.1
4,000	1.0	.1

Similar results fully confirming these were obtained by Alice L. Davison working with a more dilute solution of carbon dioxid in water.

Experiments were now carried on to determine how rapidly acetic acid is removed from its dilute solution on aerating. A solution of C. P. acetic acid in aerated distilled water was prepared, containing about 0.17 gram of acetic acid per 100 cc, that is, 10 cc of this solution was neutralized by 28.4 cc of

hundredth-normal sodium hydroxid. The results obtained on aerating 10 cc portions of the dilute acid are as follows:

TABLE 5.—*Removal of acetic acid from dilute solutions by aeration.*

Volume of air used.	Time.	N/100 sodium hydroxid.
cc.	*Minutes.*	*cc.*
2,000	1	28.1
2,000	1	28.2
12,000	4	28.0
12,000	4	28.0

Stronger solutions of acetic acid were found to lose perceptible amounts of acid on long-continued washing with air, but the losses on aerating sufficiently to remove carbon dioxid were insignificant. Aerating to remove carbon dioxid before titration will be of special value in factory and field work, particularly in the fermentation industries.

SUMMARY.

(1) The cooperative work shows that the difference between the amounts of acid indicated by litmus and by phenolphthalein, respectively, is about 20 per cent. As this difference is greater than could be accounted for by the failure of litmus to indicate all of the acid present, it is recommended that its causes be made the subject of special study.

(2) In the study of 15 fruit juices the difference between the results yielded by litmus and phenolphthalein were found to average 8.7 per cent, with a maximum of 28 per cent and a minimum of 1.8 per cent.

(3) In the titration of fruit juices using litmus solution, it is shown to be unnecessary to dilute the sample or to heat it to boiling unless the juice remains strongly colored on neutralizing (when it is well to dilute it somewhat) or contains carbon dioxid, when it is necessary to heat to boiling or wash with air to remove this gas.

(4) Carbon dioxid is readily removed from solution by washing with a current of air. A dilute acetic acid solution lost practically no volatile acid on aerating. It is recommended, therefore, that the method of aerating to remove carbon dioxid be studied in connection with the determination of total acid in fruit juices and in other products.

SOME INVESTIGATIONS CONCERNING THE KEEPING QUALITIES OF SUGAR SIRUPS, FRUIT SIRUPS, AND CRUSHED FRUITS.

By H. E. BARNARD.

It has for some time been the practice in the food and drug laboratory of the Indiana State board of health to study during each year some trade problem for the purpose of securing information and obtaining results of practical value to the men who are engaged in the preparation and distribution of food and drug products. The work done this year was suggested by the president of the Indiana Pharmaceutical Association as a subject of great importance to druggists and others operating soda fountains. Our inspectors have found the soda-fountain problem a serious one, as many fountains are insanitary, are not supplied with proper means for refrigeration, and are run by unskilled men.

The modern soda fountain with all its accessories is not easily operated, yet its management is often intrusted to an inexperienced boy. The character of the products served is not conducive to cleanliness, since the base of nearly all soda beverages is sugar sirup, causing a stickiness of utensils, draft tubes, and counters; furthermore, as the tendency of all dilute sugar solutions is to undergo alcoholic and later acetic fermentations, the man behind the carbonator has no easy task to serve only clean and acceptable drinks. The rapid increase in the number of staple fountain preparations designed to please the public taste has made it impossible for the dispenser to prepare all the goods he serves, and to-day a large part of the sirups, crushed fruits, root beers, and other much-used ingredients are bought from the manufacturers in glass, tin, or earthen jugs and kept in stock until used. It has been the practice of sirup and crushed fruit packers and canners to put up their goods in a heavy sugar sirup, and then, in order to prevent the possibility of spoilage, to add some chemical preservative. Our experiments have been carried on for the purpose of determining whether or not preservatives other than sugar are necessary, and also the conditions under which crushed fruits, fruit sirups, and fountain preparations should be handled.

The scientific study of many practical questions often falls far short of producing results because of too technical treatment. We have endeavored, however, to follow exactly the methods used at the ordinary well-operated fountain, and feel sure that every result obtained can be duplicated by a careful dispenser. The experiments have been made with a modern so-called iceless fountain, so arranged that all the refrigeration is produced by ice used in packing the ice cream buckets, one of which is at either end of the fountain.

The first problem to be studied was that of determining the most satisfactory strength of sugar solution to employ in diluting the concentrates. The sugar employed was what is known as "Crystal A," and various sirups were made from it containing 8, 10, 12, 14, and 16 pounds of sugar to the gallon of water. The sirups were prepared both by the hot and by the cold process. In the hot process, undoubtedly the best way of preparing sugar solutions, the water was heated to boiling and the sugar slowly added, stirring until solution was complete. In the cold process the sugar was added directly to water, with stirring. In all of the work distilled water has been employed. The kind of water employed is, of course, immaterial, in so far as the character of the product is concerned, if the dispenser is careful to use a pure water free from mold spores and harmful bacteria. Although five strengths of sirups were made up, yet in most cases the 10 and 14 pound solutions were used. The sirups so prepared have been kept both in the refrigerator and at room temperature. Up to this time no appreciable difference has been observed in the keeping qualities of the sirups. In no instance have the sugar sirups fermented or soured, although several lots of 10 and 12 pound sirups made up cold have, after standing for several weeks developed on the top a fine greenish-white mold, evidently a *Penicillium*. These growths have never appeared in sirups containing 16 pounds of sugar to the gallon, and in but one instance in a 14-pound sirup.

The crushed fruits used in the experiments were obtained from three sources. One lot was made up at the laboratory from fresh fruit purchased on the Indianapolis market, using the usual formulas employed at the fountain, that is to say, 2 parts of fresh fruit with its juice to 1 part of sugar. Two other lots were tinned goods sold in the State for soda-fountain purposes. The fourth lot was a well-known brand of goods prepared especially for use at the fountain.

For the purpose of studying the different kinds of fruit preparations, pineapples, strawberries, raspberries, cherries, sliced peaches, and a mixture known as "walnut sundae" were selected. At first these goods were prepared both

with and without benzoate of soda; but it became necessary to rush the work, and the investigations as to the keeping qualities of preserved goods were not long continued. It seemed self-evident that if the goods without preservative remained in good condition, those containing 0.1 per cent of benzoate of soda would certainly keep under the same conditions. The crushed fruits were prepared for use at the fountain by adding 1 part of the concentrated fruit to 2 or more parts, as required, of the simple sirup of the strengths specified, usually, however, employing only two solutions, namely, the 10 and the 14 pound mixtures. After using part of the concentrates, the remaining portion was placed in unsealed but stoppered jars in the refrigerator of the fountain, where they remained at a temperature varying from 40° to 50° F. for from one to three months. Several lots which were nearly all used were allowed to remain outside of the refrigerator at room temperature, and to our great surprise, these concentrates neither fermented nor grew moldy, but through loss of water by evaporation eventually solidified. In the endeavor to grow certain types of molds, which it was supposed would readily develop in the hot, moist air of the laboratory, various fruits, sirups, and crushed fruits were exposed in open-topped petrie dishes. It is interesting to note that goods so exposed, although open to contamination, have refused absolutely to develop molds.

Since the chief complaint of dealers has been that the concentrates, both fruit and sirup, spoil shortly after being opened and must therefore be thrown away, the keeping qualities in these goods have been carefully studied. Concentrated fruit sirups manufactured by three different houses were kept in the refrigerator at a temperature ranging from 40° to 50° F. in no case less than forty-nine days. They were then thrown out at different times ranging from forty-nine to eighty-five days after being placed in the refrigerator, not because they showed any indication of spoilage, but in order to make room for other work. It should be noted that two or three of the brands studied, including such sirups as lemon, pineapple, peach, strawberry, and raspberry, were in tin cans, and it was therefore impossible to close the tops except by pushing down the ragged-edged covers. A similar study was made on concentrated crushed fruits produced by five manufacturers, as well as on goods made at the laboratory. These products were also kept in the refrigerator of the fountain at a temperature ranging from 40° to 50° F. Twenty-seven different lots of pineapple, cherry, strawberry, walnut sundae, peach, raspberry, and chocolate were kept until they showed evidence of fermentation or mold, or, in thirteen cases, until the stock was used up. The longest period between the opening of the can and the beginning of fermentation was sixty-four days, and the shortest period was nine days, at the end of which time in the latter case a slight mold developed on the top of the product, a sample of strawberries purchased on the local market, which had been crushed with sugar and put up cold; that is, without any heat being applied to sterilize the product. Our results show conclusively that crushed fruits can be satisfactorily preserved at a temperature not higher than 50° or 55° F.

During an extremely hot period in the month of August when the room temperature was between 80° and 85° constantly, a series of experiments was run on concentrated crushed fruits to determine their keeping qualities at room temperature. The maximum period during which the goods remained free from fermentation was seven days. The minimum period was three days, at the end of which a mold developed on the surface of a raspberry crushed fruit.

The crushed fruits, prepared as just described by dilution with sugar sirup, were then placed in small glass bowls, such as are ordinarily used at the fountain, covered with a loosely fitting lid, and handled in the following ways: The first lot was kept at room temperature all the time; the second lot was

kept at room temperature for sixteen hours, and then in the refrigerator of the fountain at a temperature averaging from 40° to 50° F. for eight hours; the third lot was kept in the crushed-fruit jars, which constitute part of the fountain equipment, at a temperature ranging from 47° to 63° F. In other words, we have endeavored to treat the crushed fruits just as an ordinary dispenser treats them, employing in one instance no precaution for keeping the goods and in the other cases such precautions as would ordinarily be exercised by the intelligent operator. In but one instance in the course of these experiments with 180 samples, which were started on the 16th day of April (a period of about four months), have molds of any kind developed in the crushed fruits. The only change in the character of these products has been the development of alcoholic and acetic fermentation. As soon as any fermentation was noticeable, as evinced by the evolution of carbon dioxid, the goods were marked unsalable, although in ordinary practice there is no reason why they could not have been used for a much longer period. The following results refer only to the periods in which the goods kept in an absolutely perfect condition.

The first lot of crushed pineapple, made up with a simple sirup containing 10 pounds of sugar to the gallon, kept for twenty days in the fountain fruit jars and for seventeen days when exposed at room temperature for sixteen hours and kept in a refrigerator for eight hours.

The first lot of strawberries, made up with 10-pound sugar sirup and handled under the same conditions as the crushed pineapple, kept in each instance twelve days; when a 14-pound sirup was used they kept fourteen and eighteen days, respectively. Fountain jars prepared with 10 pounds of sugar sirup kept ten and fourteen days and with 14-pound sugar sirup twelve and eighteen days, respectively. Fountain jars prepared with 10 pounds of 68.3° to 73.9° F. and the air was very moist. None of these fruits were exposed at room temperature all the time, but the next series of experiments included such a study.

The first lot of crushed pineapple, made up at the laboratory from the raw fruit as before described and prepared with a 10-pound sugar solution, kept for seven days when exposed for sixteen hours at room temperature and kept for eight hours in the refrigerator. The goods in the crushed-fruit bowls in the fountain kept for two and one-half days. Goods made up of the 14-pound sugar solution kept approximately twice as long. Crushed strawberries treated in a similar way and made up with similar solutions kept seven and one-half days when exposed at room temperature sixteen hours and refrigerator eight hours, ten and one-half days in the fountain jars, and three and one-half days at room temperature all the time. When a 14-pound sugar solution was used under similar conditions of temperature and care, they kept eight and one-half days, fifteen days, and five days. Pineapple put up in tins when studied in this same way kept eight days, thirteen days, and five days in 10-pound sugar-sirup solution, and nine days, twelve days, and six days in 14-pound sugar-sirup solution. Strawberries put up in glass kept nine days, twelve days, and six days in 10-pound sugar solution, and in a 14-pound sugar solution, eleven days, fourteen days, and seven days. Sliced peaches put up in glass kept in 10-pound sugar solution nine days, fourteen days, and five days, and in 14-pound sugar-sirup solution eleven days, sixteen days, and six and one-half days.

Walnut sundae made up with 10-pound sugar sirup kept at room temperature seven days, and when placed in the refrigerator for part of the time for twelve days. The same goods put up with 16-pound sugar sirup kept twelve days ex-

posed at room temperature and twenty-seven days when held for a third of the time in the refrigerator.

The study of the keeping qualities of preserved and unpreserved concentrated fountain sirups was carried on by taking definite portions of the usual concentrated fruit sirups, such as lemon, pineapple, peach, raspberry, and strawberry, and making up to the proper strength by adding 1 part of the sirup to 2, 3, or 4 parts of sugar sirup. The solutions as prepared were placed in the ordinary jars provided for that purpose in the fountain. The sugar solutions used varied in density from 10 to 16 pounds of sugar to the gallon, and were made both by the hot and cold process. A definite portion was drawn by the pump from the jars each few days, the acidity of the solution determined, and the character of the sirup noted. The temperature of the sirup jars varied somewhat during the course of experiment, ranging from 53.1° to 70.0° F., the average temperature, however, being approximately 65° F.

The first sirup to be studied was the pineapple preserved with benzoate of soda and made of sugar sirup at 10, 14, and 16 pounds to the gallon. After keeping these sirups for fifty-four days at fountain temperature and observing no change whatever in their character, the solutions were discarded to make room for other experiments. Pineapple sirup containing no preservative was placed in the jars at the same time and studied for a similar period of fifty-four days. The acidity of the first solutions made up with 10 pounds of sugar to the gallon in the cold increased rapidly after the tenth day. The second sirup, however, which was made up hot with a sugar sirup of 10 pounds to the gallon, showed no increase in acidity until after the eighteenth day. The third sirup made up in a sugar solution of 16 pounds to the gallon, showed no decided increase in acidity until after the thirty-fifth day. In other words, all of the pineapple sirups kept perfectly for at least ten days after being made up, and when of a greater concentration than 10 pounds of sugar to the gallon kept for a much longer period.

Similar series of experiments were made with lemon sirups, using three unpreserved sirups and three sirups preserved with benzoate of soda. In carrying the investigation forward as in the case of pineapple sirup, the results obtained were somewhat different, however, as at the end of the fiftieth day practically no change in acidity was apparent in either the unpreserved or the preserved goods.

The results here given are for but a few of the total number of experiments run during the course of the investigation, which covered a study of 296 crushed fruits, fruit sirups, sugar sirups, and concentrates. The results briefly summarized indicate, first, that concentrated crushed fruit and fruit sirups may be kept without loss for from one to three months after opening, when held at a temperature below 50° F.; second, that fountain sirups made with 14-pound sugar sirup will keep from two to four weeks without the slightest evidence of fermentation; third, that crushed fruit concentrates diluted with sugar sirup of 14 pounds to the gallon will keep when exposed at room temperature from three to ten days, and when such goods are placed in the refrigerator of the fountain during the night for a period of eight hours the time during which they keep in good condition is nearly doubled; fourth, the keeping quality of crushed fruits and fruit sirups is influenced materially by the concentration of the sugar solutions used as diluents. While in most instances a 10-pound sirup is sufficient to hold the goods for a period long enough to allow of their disposal, yet concentrates which are from 12 to 16 pounds materially improve the keeping quality of the goods. From these experiments it appears that a 14-pound sugar solution is best adapted for use, although a saturated sugar sirup which contains about 16 pounds to the gallon of water can always

be employed with good results; fifth, it is apparent from these investigations that a sugar sirup may best be prepared by dissolving the sugar in hot water as follows: Bring the water to boiling and pour into it while stirring the number of pounds of sugar which it is desired to use. If tap water or unsterilized water is used, the sirup should be brought to 100° C. While sugar sirups made up in the cold are in most cases satisfactory, yet it is evident that they may contain mold spores, which injure the keeping quality of the preparation.

REPORT ON WINE.

By Julius Hortvet, *Associate Referee.*

On March 20 the referee on wine sent out the following letter, accompanied by methods of analysis for total, volatile, and fixed acids, and an outline for the examination of the coloring matter of wine. There were also given suggestions for work on the methods for determining glycerol. On May 15 the instructions were supplemented by a further letter giving more detailed directions for determining the acids.

Through the courtesy of Mr. William B. Alwood, of the Enological Laboratory, Bureau of Chemistry, United States Department of Agriculture, at Charlottesville, Va., the collaborators have been furnished samples of wines of undoubted purity and good quality. These samples were described by Mr. Alwood, as follows:

This case contains two samples of clarets, Nos. 4 and 5, which were made at this station and are absolutely pure. They vary only slightly in chemical composition and are from the same source; i. e., Norton's seedling grape.

There are three other purchased samples presumed to represent fairly these types of wines as made in the eastern United States. One is a so-called "port," another a Burgundy type, and the other a representative white wine called "Moselle." All of these commercial wines have been gallized, but are sound, and have not been artificially colored or chemically preserved. The samples sent are typical of sound American wines. They are all still, and if kept in a cool room, the bottle lying flat on side, will not change in character or composition.

Directions for Cooperative Work on Wine, 1909.

PREPARATION OF SAMPLE.

In order to prepare a wine for analysis various methods have been proposed. The one commonly given directs that the sample be thoroughly shaken in an open flask or poured for a short time from one flask to another. Such methods may suffice with wines having a low gaseous content, but with wines fermented under pressure, and with carbonated liquors especially, a more effective method is required, as, for example, heating the sample in an open dish to incipient boiling, or boiling under a reflux condenser. This last method is recommended for trial in connection with the determination of the acid constituents.

TOTAL ACIDS.

Measure 10 cc of wine into a 300 cc flask, add 200 cc of distilled water, and boil three minutes under a reflux condenser. After cooling, add 2 drops of phenolphthalein and titrate the solution with decinormal sodium hydroxid. In the same manner boil and titrate 210 cc of distilled water and calculate the total acids from the difference between the results of the two titrations.

VOLATILE AND FIXED ACIDS.

The apparatus (see fig. 1) consists of a 300 cc flask provided with an elongated wide neck into which is fitted a 200 cc cylindrical-shaped flask. About one-third way down the side of the latter flask is a small opening leading inward and downward through a 3 mm tube, which terminates at a point close

to the bottom of the flask. In the neck of the inner flask is also fitted a small funnel with stopcock and a delivery tube with safety bulb leading to a condenser. The distillate is received in a 100 cc cylindrical graduate.

Pour 150 cc of distilled water into the larger flask, tightly fit the inner flask into the wide neck by means of a short section of rubber tubing, run in 10 cc of wine (previously freed from carbonic-acid gas), close the stopcock, and heat the water to boiling. In extreme cases add to the wine a 0.3 gram piece of paraffin in order to diminish foaming. When the water has boiled a moment close the side tube leading out from the neck of the larger flask and pass steam through the wine until 60 or 70 cc of distillate have passed over. Empty the distillate into a beaker and continue the distillation. Titrate the distillate with decinormal sodium hydroxid, using phenolphthalein as indicator. Stop the distillation when an additional 10 cc of distillate requires not more than one drop of the standard alkali solution to neutralize. Usually 80 to 90 cc of distillate will include practically all of the volatile acids.

On cooling the apparatus, the wine liquor is siphoned into the lower flask. Rinse out the remaining liquor by running in a couple of small portions of hot water through the funnel tube; then disconnect the two flasks. In the case of

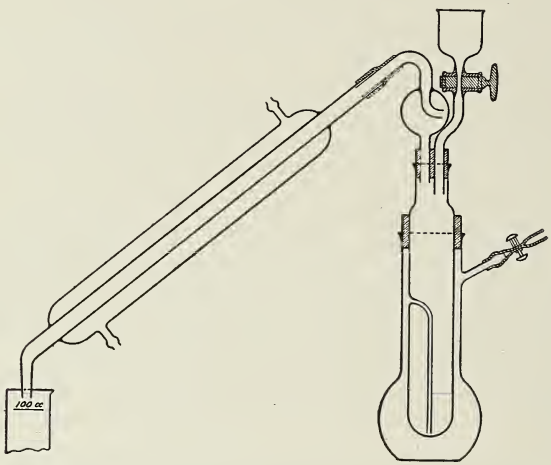

FIG. 1.—Apparatus for determining volatile and fixed acids.

a white wine or a light-colored wine, add 100 to 200 cc of distilled water and titrate the fixed acids with decinormal sodium hydroxid, using phenolphthalein as indicator. In the case of a highly colored wine, cool the liquor, make up to 100 cc, measure out 25 cc, dilute with distilled water, and titrate as before.

TOTAL, FIXED, AND VOLATILE ACIDS.

[Supplemental directions, May 15, 1909.]

In titrating total acids according to the proposed new method, use a 500 or 600 cc flask for the boiling under a reflux condenser, and after boiling rinse out the condenser tube with a little distilled water and, in case of a highly colored wine, add 100 or even 200 cc of water. Use 3 or 4 drops of phenolphthalein solution if deemed necessary and titrate carefully, adding the standard alkali slowly toward the last and continuing until the change in color produced on adding 2 or 3 drops of the alkali is hardly perceptible. The titrations can be carried out successfully in good light over a white surface, holding the flask a short distance above the table. After preliminary trials, make two determinations and report the mean of the results. Proceed in a similar manner in titrating the fixed acids remaining in the apparatus after the volatile acids have been distilled off. It will be best to transfer the liquor and dilute in the same flask that is used in the titration of the total acids. In distilling off the volatile acids by the new method, I have often found it necessary to collect

100 cc of distillate. Distil in all cases until the last 10 cc of distillate appears to require practically no more of the alkali for neutralization.

Read referee's article in the Journal of Industrial and Engineering Chemistry, January, 1909, pages 31–38, and make the color examinations after the acid determinations have been completed.

EXAMINATION OF THE COLOR.

Make all color comparisons and descriptions with reference to the Bradley color standards employed by S. P. Mulliken in his book entitled "A Method for the Identification of Pure Organic Compounds." A description of the standards and a discussion of the application of color reactions to the examination of unknown substances are given on pages 230 to 234.

In matching colors the best results are obtained if the observer stands with his back toward a window and not in direct sunlight. The material to be examined should be held about an inch away from the white cardboard accompanying the charts and alongside the square opening in the cardboard. Move the cardboard about until the exposed color matches as nearly as possible the color of the material. Make the tests on the undiluted sample unless otherwise stated. In tests with the various reagents note the color of the solution as well as of the precipitate. In the solubility tests with amyl alcohol and ether note the color imparted to the solvent as well as that remaining in the wine.

(a) Pour into each of six test tubes about 5 cc of the sample and apply the following solubility tests: To each of two portions, one acidified with a few drops of hydrochloric acid and one made alkaline with ammonia, add 5 cc of ether. To each of two portions, made respectively acid and alkaline in the same manner, add 5 cc of amyl alcohol. Shake the tubes thoroughly and allow the liquids to separate. If in any case an emulsion is formed, add a few drops of ethyl alcohol. Make similar tests on the remaining portions of the sample without the addition of acid or alkali.

(b) Make 10 cc of the sample alkaline with baryta water, shake with an equal volume of amyl alcohol, and, after observing the colors of the two layers as directed, add acetic acid to a filtered portion of the amyl alcohol layer.

(c) To 50 cc of the wine in a beaker add an equal amount of water and a few cubic centimeters of dilute hydrochloric acid. Place in this a piece of white, fat-free wool cloth, about 10 cm square, and boil the solution from five to ten minutes. Remove the cloth, wash in a stream of water, and, after noting the color, treat the wool with a 2 per cent ammonia solution.

(d) Add 5 cc of concentrated nitric acid to an equal amount of wine in a test tube.

(e) To 10 cc of the wine in a test tube add 5 cc of a neutral or slightly alkaline mixture of 10 per cent potassium-alum and 10 per cent sodium carbonate, shake the mixture, and separate the precipitate by filtering. In like manner test the wine with 5 cc of a 10 per cent aluminum acetate solution made alkaline with 10 per cent sodium carbonate solution. Treat a portion of the sample also with a saturated solution of mercuric chlorid.

(f) To 15 cc of the wine in a test tube add 3 cc of a 10 per cent solution of lead subacetate. Shake the solution and filter.

(g) Add about 0.50 gram of pulverized yellow oxid of mercury to 20 cc of the sample, heat the mixture to boiling, cool, and pour through a double filter. After first noting the color of the filtrate, add a few drops of hydrochloric acid.

(h) To each of two 10 cc portions of the sample in test tubes add respectively a few drops of 10 per cent solution of ferric chlorid and a few drops of 10 per cent solution of ferrous sulphate.

Express the results in terms of the following abbreviations: R=red, OR=orange-red, RO=red-orange, O=orange, YO=yellow-orange, OY=orange-yellow, Y=yellow, GY=green-yellow, YG=yellow-green, G=green, BG=blue-green, GB=green-blue, B=blue, VB=violet-blue, BV=blue-violet, V=violet, RV=red-violet, VR=violet-red, t_1=tint one, t_2=tint two, s_1=shade one, s_2=shade two, sol=soluble or solution, ppt=precipitate, filt=filtrate, bt=broken tone. Whenever it seems impossible to match colors with the chart use general terms, as blue-gray, brown-gray, etc.

GLYCEROL.

No method is offered in place of the provisional method for glycerol, as given in Bulletin 107, Revised, page 83. Collaborators are requested to examine other methods with a view to proposing a new method for study in connection

with future work. The following references are offered: Method of C. Billon, Sixth International Congress of Applied Chemistry, Rome, 1906; J. Soc. Chem. Ind., 1906, **25**:779; Analyst, 1903, **28**:75; A. A. Shukoff and P. J. Schestakoff, Zts. angew. Chem., 1905, **18**:294–295; J. Soc. Chem. Ind., 1905, **24**:294; Analyst, 1905, **30**:160; Franz Zetzsche, Pharm. Zentralhalle, 1907, **48**:797, 803, 820; Zts. Nahr. Genussm., 1908, **15**:117; Chemical Abstracts, 1907, **1** [24]:3040.

Following the foregoing directions and also those given in Bulletin 107, Revised, Bureau of Chemistry, U. S. Department of Agriculture, carry out the work outlined below on the samples submitted for collaborative work. Plan the work so as to complete the acid determinations within a day or two, in the meantime keeping the samples in a refrigerator, thus avoiding changes in composition.

1. Determine:
   - Total acids—
     - (a) By the provisional method of the association.
     - (b) By the proposed new method.
   - Fixed acids—
     - (a) By the provisional method of the association.
     - (b) By the proposed new method.
   - Volatile acids—
     - (a) By the provisional method of the association.
     - (b) By the proposed new method.

Express all results for total, fixed, and volatile acids in terms of the number of cubic centimeters of normal acid in 100 cc of wine.

2. Apply the tests for color as described in the foregoing outline, and tabulate the results in the order in which the tests are given.

Cooperative Work on Acidity.

Total, fixed, and volatile acids in wines.

[Results expressed as cubic centimeters of normal acid in 100 cc wine.]

NORTON CLARET No. 4.

Collaborators.	Total acids.		Fixed acids.		Volatile acids.	
	By provisional method.	By new method.	By provisional method.	By new method.	By provisional method.	By new method.
J. R. Eoff, jr., Charlottesville, Va.	9.00	9.95	7.56	[a]8.07	1.44	1.70
Azor Thurston, Grand Rapids, Ohio	9.60	9.80	8.06	7.60	1.54	1.45
W. F. Sudro, Fargo, N. Dak.	8.70	9.60	6.54	7.85	2.16	1.80
H. C. Gore, Washington, D. C.	8.99	10.20	7.57	8.70	1.51	1.63
Edmund Clark, Boston, Mass.	10.60	11.00	9.10	8.90	1.50	1.60
Julius Hortvet, St. Paul, Minn.	8.60	10.20	7.10	8.60	1.50	1.60

NORTON CLARET No. 5.

J. R. Eoff, jr.	9.34	9.91	7.41	[a]7.71	1.93	2.37
Azor Thurston	9.80	10.20	7.72	8.00	2.08	2.20
W. F. Sudro	9.15	9.75	6.08	7.35	3.07	2.25
H. C. Gore	9.32	10.10	7.21	8.70	2.11	2.15
Edmund Clark	11.00	11.20	8.88	8.93	2.12	2.25
Julius Hortvet	8.80	11.60	6.84	9.50	1.96	2.20

PORT TYPE.

J. R. Eoff, jr.	9.53	10.05	7.85	[a]8.08	1.67	1.82
Azor Thurston	10.60	10.40	8.86	8.00	1.74	1.70
W. F. Sudro	9.05	9.90	7.23	7.95	1.82	2.00
H. C. Gore	9.34	10.30	7.62	8.60	1.72	1.85
Edmund Clark	10.58	10.30	8.95	8.60	1.63	1.65
Julius Hortvet	9.20	11.30	7.50	9.50	1.70	1.75

[a] Fixed acids determined by taking residue from jacket flask, making to volume of 100 cc and titrating aliquot portion (25 cc=2.25 cc original wine). All other fixed acid results obtained by titration of entire residue.

Total, fixed, and volatile acids in wines—Continued.

[Results expressed as cubic centimeters of normal acid in 100 cc wine.]

BURGUNDY TYPE.

Collaborators.	Total acids.		Fixed acids.		Volatile acids.	
	By provisional method.	By new method.	By provisional method.	By new method.	By provisional method.	By new method.
J. R. Eoff, jr	9.33	10.02	7.69	[a] 7.77	1.63	1.88
Azor Thurston	9.32	9.80	7.54	8.00	1.78	1.80
W. F. Sudro	9.00	9.65	6.49	7.55	2.51	2.10
H. C. Gore	8.94	9.80	7.25	8.80	1.69	1.93
Edmund Clark	10.88	10.10	9.11	.....	1.77	.....
Julius Hortvet	9.00	10.80	7.26	9.00	1.74	1.85

WHITE WINE (a Moselle).

J. R. Eoff, jr	10.01	10.44	8.92	[a] 9.10	1.09	1.30
Azor Thurston	10.40	10.40	9.04	8.80	1.36	1.30
W. F. Sudro	9.40	10.25	8.09	8.98	1.31	1.18
H. C. Gore	9.80	10.20	8.42	8.83	1.38	1.40
Edmund Clark	11.08	10.25	9.62	.....	1.46	.....
Julius Hortvet	9.68	10.60	8.42	9.10	1.26	1.40

[a] Fixed acids determined by taking residue from jacket flask, making to volume of 100 cc, and titration aliquot portion (25 cc=2.25 cc original wine). All other fixed acid results obtained by titration of entire residue.

COMMENTS OF ANALYSTS.

J. R. Eoff, jr.: New methods: In the volatile acid determinations in all cases it was possible to titrate to the fraction of a drop of tenth-normal alkali and to read the burette to 0.01 cc. The volumes of distillate collected, which contained all of the volatile acid, were as follows:

Norton No. 4, 110 and 90 cc; Norton No. 5, 90 and 100 cc; Port, 90 and 90 cc; Burgundy, 100 and 100 cc; and Moselle, 90 and 80 cc. The time required for the completion of the volatile acid determination varied from twenty-five to twenty minutes.

In preliminary trials it was found that the size of gas flame used greatly influenced the volume of liquid in the inner flask of the Hortvet apparatus, with a corresponding variation in the result. It was found that the flame of a good alcohol spirit lamp gives the most satisfactory results, scarcely any increase being noticed in the volume of wine. The data given below show the variations in the result, on test runs, with a single sample of wine and a known acid solution.

Comparison of tests using acetylene and alcohol flames.

Number of test.	Intense acetylene flame (2.5 inches).	Alcohol burner.	
	No. 1 Norton. (Normal acid per 100 cc wine.)	No. 1 Norton. (Normal acid per 100 cc wine.)	10 cc N/50 acetic acid (N/10 alkali).
	cc	*cc*	*cc*
1	2.30	2.64	1.96
2	2.67	2.66	[a] 2.00
3	2.35	.....	[b] 2.00
4	2.76	.....	.....

[a] Normal alkali. [b] Theoretical.

With the Moselle wine no difficulty was experienced in the titration following the proposed new method for fixed acids, but with the remaining four wines no satisfactory end point could be discerned when the directions given were followed. However, by slight modifications of the process, this difficulty was readily overcome, namely, for Port wine and Burgundy: By using 500 cc of recently boiled and neutralized water and 5 drops of phenolphthalein solution, and then titrating in a large white porcelain dish a very satisfactory observation of the end point could be made.

With Norton Nos. 4 and 5 further difficulty was experienced. Even with the use of the large dish no end point could be observed. It was found that by adding about 20 drops of a 0.5 per cent solution of phenolphthalein to the solution of wine in the dish at a point in the titration when the solution became distinctly green, a very distinct end point could be observed. The results reported were obtained by the method given above.

It will be noticed in the tabulated results that the fixed acids in the dark colored wines were in part determined by using the entire residue remaining in the jacket flask after the distillation of the volatile acids, and in part by using an aliquot of same. In all cases the results obtained by using the aliquot were lower than when the total residue was employed. Two hundred cubic centimeters of water were used when the aliquot was titrated. It seems inadvisable to use an aliquot portion, since any error will be so greatly multiplied, and it is fully as easy to use the entire residue. I would deem it best to dispense with the direct determination of fixed acids and calculate the same from determinations of total and volatile acids on the original sample by methods given. There seems to be no practical advantage to be derived from this extra procedure, save when there is a scarcity of sample. The same difficulties were met with in determining total acids as in the case of fixed acids, and were overcome in the same manner.

Provisional methods: The method for total acid was followed, with the exception that 20 cc of wine were used, and a very sensitive solution of pure neutral azolitmin on white tile was prepared in the laboratory and substituted for litmus paper. The provisional method for volatile acid was followed exactly, but for the purpose of testing this method distillation was carried to the practical disappearance of acid in the distillate. The fixed acid was calculated from the volatile and total acids.

Volatile acid determinations on successive portions of the distillate (Provisional method).

Kind of wine.	Successive portions of the distillate.				
	First 200 cc.	Third 100 cc.	Fourth 100 cc.	Fifth 100 cc.	Total.
Norton No. 4 (claret)	1.44	0.16	0.04	.....	1.64
Norton No. 5 (claret)	1.93	.26	.10	0.06	2.35
Port	1.68	.10	.04	.....	1.82
Burgundy	1.63	.17	.03	.04	1.87
Moselle	1.09	.12	.....	.....	1.21

Azor Thurston: For volatile acids, I find about the same results, with the proposed method as with the provisional, but am very much in favor of your method, as the time necessary to complete the analysis is much shortened, and the volatile acids passed over in 70 cc of the distillate. Phenolphthalein as an indicator for the fixed acids does not appear quite satisfactory, but the same might be said of litmus.

W. F. Sudro: Determined the end point of total acids (provisional method) with blue litmus paper, titrating to neutral point. Very poor results with volatile acids determination by the provisional method. The new methods give very uniform results. End point in determination of both total and fixed acids very easily ascertained.

H. C. Gore: Total and fixed acids: The proposed method gives results from 4 to 12 per cent higher than those obtained by the provisional method. (This also probably gives results which are too high.) A valuable feature of the proposed method is the removal of the carbon dioxid by boiling under a return condenser. The reasons why phenolphthalein gives too high results are two:

First, the pink tinge of the phenolphthalein is apt to be masked at first by the greenish shades in the wine. Brown tones are produced rather than the pink. This objection is removed by running a blank, i. e., a sample containing no indicator, side by side with the sample containing phenolphthalein. A difference in shade shows the end point. Second, the titration is of necessity carried on until the solution is alkaline, and a large number of organic substances exist, among them tannin and coloring matter, which possess very slight acid characters, or are not acid at all, but still absorb alkali. Unless it is desired to include in the total acid figure the titration values of such bodies as tannin, the use of phenolphthalein will give high results. By using litmus these difficulties are avoided.

Volatile acid: The proposed method gives results which are probably more exact, though higher than those obtained by the provisional method. The apparatus is a great advance over any other form. The details of the operation are unnecessarily cumbersome, but can easily be simplified. There is another point to be considered, and that is, the danger of the distillate becoming contaminated with carbon dioxid and so giving high results. This will probably be a serious matter when working where the air is rich in carbon dioxid, as near a winery or distillery. It can of course be guarded against by protecting the distillate, or by heating it under a return condenser before titrating.

Method of expressing results: It seems to me that the proposed method of expressing results is without justification. Its use will be to make the work of chemists less useful, rather than more so, because the figures will be unintelligible except to the few, and can not readily be used by pomologists or hygienists, or even by chemists in other fields. No deception occurs in expressing the acid in terms of the acid present in largest quantities, e. g., total acid as tartaric.

Edmund Clark: I can see but a slight advantage in accuracy in the estimation of volatile and fixed acidity by the proposed method over the provisional method, and a decided disadvantage in the matter of time. I prefer the provisional method for total acids also and by running a preliminary titration to fix the acidity approximately the time of operation can be materially shortened.

COMMENTS OF ASSOCIATE REFEREE.

It was arranged this year that all of the collaborators should have wines of the same kind, and as nearly as possible of the same composition, for the purpose of providing like conditions under which to carry out the work. Each person was also provided with apparatus of the same design and construction for the study of the new methods and was given careful directions for making the determinations. While there are some wide discrepancies among the results, the general plan of the work appears to have been carefully carried out in nearly all cases, and it is possible to arrive at some conclusions. With only a few exceptions, among the sixty determinations of total and fixed acids, the results obtained by the proposed new methods are higher than those obtained by the present provisional methods. This is fully as true in the case of the white wine as in the case of all of the colored wines. One collaborator reported results, which, for the reasons stated in his comments, form a class by themselves, and can hardly be considered in connection with the results reported by the others. While practically all agree in obtaining higher results by the new method, they have not in most instances carried their titrations as far as the points indicated by the results given by the associate referee. These higher results, it appears, were obtained under conditions not described by the collaborators, and which consist chiefly in the fact that the titrations were carried out in a large flask held in good light a short distance above a white tile surface; hence it follows that the end points of the titrations were judged by light transmitted from below. The exact point of neutrality was judged when under conditions of good light the addition of the standard alkali failed to cause any further marked change due to the phenolphthalein. Changes occurring in the wine when diluted to 400 or 500 cc were carefully noted, and have been found in general to correspond with those described by H. C. Gore. The discrepancies

noted among the results doubtless arise chiefly on account of differences in the manner of judging the point at which the titrations are completed. Much greater differences appear among the results obtained when litmus was used as an indicator. This is doubtless due largely to inherent defects in the indicator, a fact that was pointed out in the report presented last year. The fact is, however, apparent that in order to secure concordant results with either indicator, certain strict conditions must be agreed upon. The following method of titrating with phenolphthalein given by Gore[a] is deserving of consideration:

If phenolphthalein is present its effect at the time the end point for this indicator is reached, is slightly to change the shade of the solution. This occurs just after the green begins to develop. As the titration progresses from the point where the green color first appears, the green deepens, and at the same time the red, due to the plenolphthalein, begins to develop and it is impossible to detect with certainty at just what point a change in color, due to phenolphthalein, occurs unless the somewhat cumbersome device is employed of titrating a blank, that is, a grape juice solution to which no indicator has been added, side by side with the solution containing phenolphthalein, using another burette. The slight amount of red naturally occurring in the neutralized or alkaline grape juice probably renders the change in shade due to slight amounts of phenolphthalein red still more difficult to recognize. By titrating a blank simultaneously, however, adding exactly the same amount of alkali as employed in titrating the solution when phenolphthalein is used, the true end point may be found quite as sharply as could be desired.

From the standpoint of uniformity, the results seem to favor the use of phenolphthalein, in spite of the difficulties mentioned. Attention is also called to the method of titrating with litmus, described by Gore in the article just mentioned. This method is as follows:

The litmus used was purified by one of the methods found in the standard text-books, which directs the removal of the coloring matters other than the azolitmus by extraction with hot alcohol. In titrating, a dilute solution of litmus of neutral tint was placed in small drops on a white glass plate and was thus used as an outside indicator. This method, including the precaution of employing a litmus which has been thoroughly extracted with hot alcohol, is due to Mr. C. S. Ash, chemist of the California Wine Association. In titrating a dilute solution of an organic acid the end point is essentially the same as when the indicator is used inside. In the titration of a colored grape juice the end point is satisfactory and much sharper than by either using litmus inside or outside on paper. After exposure to the air for a few moments on the white plate the drops of neutral tinted litmus become blue, and very slight amounts of free acid in the drops of the solution being titrated are easily detected by the reddish tinge imparted. If the sample is greatly diluted—say one part of the juice to forty parts of water—somewhat low results may be expected, but there will probably be found no occasion for great dilutions, as it is found that the natural colors even of the very dark, hot-pressed Concord juices do not interfere with the sharpness of the end point, duplicates agreeing usually within 0.1 cc.

Which indicator is the correct one to use, on theoretical grounds, is a question open for consideration. The associate referee does not quite agree with Gore regarding the removal of tannin by means of carbon black before carrying out a titration on a red wine. It is not clear why any substance like tannin, which has the characteristics of an acid, should not be included among the total and fixed acids of wines. The conclusions reached by Gore on this point do not seem necessarily to follow from the results obtained. While, in general, we agree in the results obtained with the two indicators, it is not possible at present to agree to the statement that "Results on highly colored grape and other juices, and on many red wines, will probably be much too high if the latter indicator (i. e., phenolphthalein) is used."

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[a] J. Ind. Eng. Chem., 1909, 1:9.

There is a general sentiment in favor of the new form of apparatus for determining volatile acids. Nearly all results obtained with this apparatus are higher than results by the official method. Doubtless, as pointed out by the Enological Laboratory, the character of the flame, as well as the time of the distillation, are determining factors in the method.

All collaborators, with one exception, reported results in terms of normal acid in 100 cc of sample. This method of expressing results has advantages which are sufficiently apparent to require no comment, and it is hoped that food chemists will adopt it in the interest of securing uniformity, as well as for the convenience in comparing our results.

Examination of Colors.

The four reports received on examination of the color have been arranged in groups so that comparison may easily be made of the results obtained by the four collaborators on the same kind of wine. All collaborators, excepting one, agree that the natural color in wines is soluble in ether, the Enological Laboratory finding Burgundy soluble in ether under neutral conditions, giving ORt_1–OR. Mr. Eoff says: "The suggestions were carried out in full with the exceptions that the test with aluminum acetate was omitted. * * * Used a saturated solution of mercuric chlorid, as this is as near a 10 per cent solution as could be obtained. It was rarely the case that the colors exactly matched any on the chart, and the figures given are those most closely approximating the colors obtained." All note a strong reaction with nitric acid.

Results on (e) vary widely, though it may be attributed either to the condition of the first two solutions, which should be alkaline and freshly prepared, or the discrepancy may be in the fact that the color of the filtrate only is reported. Neither Sudro nor Thurston states whether the color noted is from the precipitate or the filtrate.

The filtrate from yellow oxid of mercury, when treated with hydrochloric acid, shows a coloration varying from Ot_2 to V–R. This is contradictory to reactions for normal wine given in Bureau of Chemistry Circular, No. 25, page 16.

The collaborators did not note whether ferric chlorid formed a precipitate or not. With this exception, the conclusions published in the last Proceedings (Bureau of Chemistry Bulletin No. 122, p. 17) were confirmed by the collaborators on these wines of known purity, as follows:

(1) The natural color of wine is insoluble in ether.

(2) Nitric acid destroys the color of red wines.

(3) The lead subacetate precipitates vary from pale yellow to deep blue gray, but a violet or red color is never found in the precipitate from a genuine wine.

(4) Red wines give a coloration upon the addition of hydrochloric acid to the filtrate from the yellow oxid of mercury.

Description of sample and name of collaborator.	Natural color of wine.	Solubility tests with amyl alcohol.				Wool-dyeing test.	
		Neutral.	Acid with hydrochloric acid.	Alkaline with ammonia.	Alkaline with baryta water.	First acid bath.	Action of 2 per cent ammonia.
NORTON CLARET NO. 4.							
U. S. Enological Laboratory, Charlottesville, Va.	R	Sol. Rt_2—ORt_2 deep R below	Sol. Rt_2 R—Rs below	Sol. Gt_2, Gs_2 below	Colorless +$C_2H_4O_2$ faintly pink	Rs_1	Dark gray-brown
Azor Thurston, Grand Rapids, Ohio.	R	Sol. O—Rt_2	Sol. V—Rt	Almost insoluble	Faint Y—T_2 +$C_2H_4O_2$ no change	O—Rt_2	B—Gs_1
W. F. Sudro, Fargo, N. Dak.	.....	Sol. O—Rbt	Sol. Rt_1	Sol. Y—G—Yt_2	Colorless +$C_2H_4O_2$ Y—O—OYt_2	Rbt violet tinge	Purple red
Genevieve Imus, St. Paul, Minn.	Rs_2	Sol. Rt_1 Rs_2 below	Sol. V—Rt_1 Rs_1 below	Insol.	Colorless +$C_2H_4O_2$ no change	Dull R—Ot_2	Ys_2
NORTON CLARET NO. 5.							
Enological laboratory.	R	Sol. Rt_2 deep R below	Sol. Rt_2—Rt_1 R below	Sol. tinge of green, brown-black below	Colorless +$C_2H_4O_2$ trace of pink	Rs_1	Dark gray-brown
Azor Thurston.....	O—Rs_2	O—Rt_2	V—Rt_1	Almost insol.	Ot_2 +$C_2H_4O_2$ Rt_2	O—Rt_2	B—Gs_1
W. F. Sudro.....	.....	Sol. Obt	Rt_1	Y—GYt_2	Colorless +$C_2H_4O_2$ O—Yt_2	Rbt purple	Purple
Genevieve Imus....	Rs_2	Sol. R—Ot_1 Rs_2 below	Sol. Rt_1 R below	Insol.	Colorless +$C_2H_4O_2$ no change	Dull R—Ot_2	O—Ys_2
PORT.							
Enological laboratory.	.....	Sol. Y—Ot_2 O—Rs_2 below	Sol. YOt_1—Ot_1 Rs_1 below	Sol. slightly greenish, brown-black	Colorless +$C_2H_4O_2$ trace of pink	O—Rbt	Y—Obt
Azor Thurston.....	R—Os_2	Sol. Y—Ot_2	Sol. Ot_1	Very faint brown	Colorless +$C_2H_4O_2$ no change	Dull Ot_2	B—Gs_1
W. F. Sudro.....	.....	Sol. O—Yt_2	Sol. Y—Ot_1	G—Yt_2	Colorless +$C_2H_4O_2$ Y—OYt_2	Y—Obt red tint	Y-brown
Genevieve Imus....	O—Rs_2	Sol. Y—Ot_2 Rs_2 below	Sol. Os_1 Rs_1 below	Insol.	Colorless +$C_2H_4O_2$ no change	Dull Ot_2	Ys_2

work on colors in wine.

General reactions.							
Action of nitric acid.	Potash alum+ sodium carbonate.	Aluminum acetate+ sodium carbonate.	Mercuric chlorid.	Lead sub-acetate.	Yellow oxid of mercury.	Ferrous sulphate.	Ferric chlorid.
Y—O—Y	B—Gbt ppt.	.....	V—Rs_2 ppt. V—Rs_1 —VRs_2 filtrate	B—G ppt. R—Vbt filtrate	Obt +HCl V—R	Rs_1	R—Vs_2
Yt_2	Y—Gs_2	Y—Gs_2	Rs_1	B—G ppt. colorless filtrate	Y—Ot +HCl VRt_1	No change	R—Os_2
O—Y	Rs_1	Rs_2	R violet tinge	VR—Rs_1	R—Ort_1 +HCl Rt_1 tinge of violet	Rs_1	Rs_1 violet
O—Y	B—Gs_1 ppt. G—Ys_2 filtrate	B—Gbtppt. Y—Os_2 filtrate	R—V ppt. R—Vs_1 filtrate	B—Gs_1 ppt. colorless filtrate	R—V+HCl O—R	No ppt. Rs_1 sol.	R—Os_2 ppt. deep brown filtrate
Y—OY	BGbt	.....	V—Rs_2 ppt. V—Rs_1 —ORs_2 filtrate	B—G ppt. R—Vbt filtrate	ORbt +HCl V—R	RS_1	R—Vs_2
Yt_1	Y—Gs_2	YGs_2	No change	B—G ppt. colorless filtrate	Y—Ot_2 +HCl VRt_1	No change	R—Os_2
O—Y	Rs_1	Rs_2	RO—OR	VR—Rs_1	Y—Ot_1 +HCl VR—Rt_1	O—Rs_1	Rs_1 tinge of violet
O—Y	B—Gs_2 ppt. G—Ys_2 filtrate	B—Gbt ppt. Y—Os_2 filtrate	V—Rs_2 ppt. V—Rs_2 filtrate	B—Gs_2 ppt. colorless filtrate	R—Os_1 filtrate +HCl R	No ppt. Rs_1 sol.	R—Os_1 ppt. brown filtrate
Y—OY	Y—Obt ppt.	.....	Obt ppt. Rs_1 filtrate	Brown-black ppt. Y—Os_1 —O—Ys_1 filtrate	OYt_1 +HCl Ot_2	Rs_1	Brown-black
O—Ys_1	R-brown	Dark R-brown	No change	Colorless filtrate.	O—Ys_2 +HCl opalescent Ot_2	No change	Ybt
Y	Os_1	Os_2	Os_1	O—Yt_1	O—Yt_1 +HCl YO—OYt_2	Os_1	R—O—Rs_1
O—Y	Y—Obt. ppt. Os_2 filtrate	Y—Obt ppt. O—Ys_2 filtrate	No ppt. R—Vs_1 filtrate	Ybt ppt. colorless filtrate	Colorless +HCl no change	No ppt. O—Rs_1 sol.	No ppt. black solution

Description of sample and name of collaborator.	Natural color of wine.	Solubility tests with amyl alcohol.				Wool-dyeing test.	
		Neutral.	Acid with hydrochloric acid.	Alkaline with ammonia.	Alkaline with baryta water.	First acid bath.	Action of 2 per cent ammonia.
BURGUNDY.							
Enological laboratory.	.....	Sol. Or—ORt$_1$ brown-black below	Sol. R below	.....	Colorless +$C_2H_4O_2$ trace of pink	Rbt	Light brown-gray
Azor Thurston.....	Rs$_1$	Sol. R—Ot$_1$	Sol. O—R	Nearly insol.	Colorless +$C_2H_4O_2$ no change	Ot$_2$	B—Gs$_1$
W. F. Sudro.....	.....	Sol. O—YOt$_1$	Sol. Os$_1$	Yt$_2$	Colorless +$C_2H_4O_2$ Y—OYt$_2$	O—Rbt violet tinge	G-purple
Genevieve Imus....	Rs$_2$	Sol. R—Os$_1$ Rs$_2$ below	Sol. O—R R below	Insol.	Colorless +$C_2H_4O_2$ no change	Dull R—Ot$_2$	Y—S$_2$

colors in wine—Continued.

General reactions.							
Action of nitric acid.	Potash alum+ sodium carbonate.	Aluminum acetate+ sodium carbonate.	Mercuric chlorid.	Lead subacetate.	Yellow oxid of mercury.	Ferrous sulphate.	Ferric chlorid.
Y—O—Y	B-grey ppt.	.....	Rbt ppt. Rs_2 filtrate	B—Gbt ppt. Y—Os_2 —Y—Obt filtrate	OYt_1 +HCl ORt_2	Rs_2	Muddy violet
Yt_1	G—Ybt	G—Ybt	No change	Colorless filtrate	Y—Ot_2 +HCl R—Ot_2 opalescent	No change	Ybt
Y	R—O—Rs_1	R—O—Rs_2	O—Rs_1	O—Yt_1	YO—O—Yt_1 +HCl R—O—Ot_1	R—Os_1	Rs_1
O—Y	G—Yt_2 ppt Ys_2 filtrate	G—Ybt ppt. Y—Os_2 filtrate	Rbt O—Rbt filtrate	B—Gbt colorless filtrate	R—Os_1 +HCl R—O	No ppt. Rs_1 sol.	R—Os_1 ppt. deep brown filtrate

The following report on the determination of glycerol in the official wine samples was submitted by Mr. Hartmann:

Determination of Glycerol in Official Wine Samples.

By B. G. Hartmann.

PROVISIONAL METHOD.

For the determination of the glycerol in the Moselle, Burgundy, and the two Norton wines submitted, the provisional method described in Bulletin 107 under "Dry Wines" was used, whereas in the case of the port (having about 17 per cent extract) the method described under "Sweet wines," same bulletin, was followed. In the first instance 15 cc of milk of lime were required and in the latter 40 cc were found to be necessary. The method gave little trouble. Would suggest that the ether be entirely expelled by evaporation on water bath in an Erlenmeyer flask before transferring to dish for final evaporation. This will prevent creeping and consequent loss of material.

ACID-DICHROMATE METHOD.

The standard dichromate solution of such strength that 1 cc of the solution equals 0.01 gram of glycerol was prepared by dissolving 74.69 grams of pure potassium-dichromate in sufficient water to make 1,000 cc. This weight was deduced from the formula for oxidizing glycerol into carbon dioxid and water, the available oxygen of 7 molecules of dichromate being required for the complete combustion of 3 molecules of glycerol.

After checking this solution against ferrous ammonium-sulphate and finding it to be correct, the method was tried on glycerol directly. Purest glycerol was dried for two and one-half hours in a water oven and 12.3402 grams made up to 500 cc. Twenty cubic centimeters of this solution corresponding to 0.4936 gram were used and the following results on two determinations were obtained: Test I, 0.4761; test II, 0.4757. Expressing the amount of glycerol recovered in percentage, it was found that the method yielded 96.5 per cent of glycerol. Considering the probable impurity of the glycerol on the one hand and the high concentration of the solutions on the other, the accuracy of the dichromatic solution as well as the reliability of the oxidation formula must be admitted. The following are the results obtained by both methods:

Glycerol determinations by two methods.

[Grams per 100 cc.]

Kind of wine.	Provisional method.			Acid-dichromate method.		
	Glycerol.	Average.	Ash in residue.	Glycerol.	Average.	Difference between methods.
Norton No. 4	0.928 .934	0.931	0.043	0.730 .774	0.752	0.179
Norton No. 5	.934 .933	.934	.046	.735 .763	.749	.185
Port	.478 .475	.477	.005	.392 .392	.392	.085
Burgundy	.655 .683	.669	.011	.568 .565	.567	.102
Moselle	.674 .685	.680	.020	.585 .576	.581	.099

The table shows that the acid-dichromate method gives lower results than the provisional method by from 0.1 to 0.2 per cent. Furthermore, it has been shown that highly purified glycerol dissolved in water will yield 96.5 per cent of the glycerol content when using the acid-dichromate method. Is the residue obtained by the provisional method truly glycerol? It seems doubtful. and the suggestion is made that the residue be tested for organic impurities soluble in water.

The water-insoluble glycerol residue was determined by treating the glycerol residue with hot water, filtering, washing with hot water, igniting, and weighing. Comparing these results with those given by igniting the residue and weighing the ash, the following figures were obtained: For Moselle, ash in residue 0.014 gram, and water-insoluble portion 0.002 gram. For port, ash in residue 0.011 gram, and water-insoluble portion 0.001 gram.

DETERMINATION OF GLYCERIN IN WINE.

By S. H. Ross.

In the report given by the referee[a] on this subject last year the work was done on unknown samples, and, as stated, a loss of glycerin took place during the determination which could not be estimated; further, the material which was extracted and weighed as glycerin by the provisional method was not pure glycerin, but contained from 85 to 88 per cent, as the referee found by subsequent oxidation of the water-soluble residue.

Before making this determination on wine proper, it seemed desirable to work on imitation wines, which contained known amounts of glycerin. Since the loss of glycerin which takes place during the determination by the official method can not be estimated, an effort was made to modify the method in order to eliminate certain losses, especially those which occur in appreciable amounts in the preliminary evaporation, if conducted at a high temperature (above 90° C.), and in the final drying for one hour at the temperature of boiling water. From an imitation wine 95.65 per cent of the glycerin present was recovered. The loss of 4.35 per cent may be reduced, but since the method at best involves numerous evaporations, filtrations, washings, and transfers, the natural mechanical loss can not be entirely eliminated, even with extremely careful manipulation. If, however, a chemist operating on known samples recovered uniformly about 96 per cent, he would be warranted in assuming that similar results would be obtained on unknowns or wines proper.

Modified Provisional Method for Glycerin in Dry Wines.

The provisional method[b] was modified to read as follows:

EXTRACTION.

Make all evaporations on a water bath, at a temperature between 85° and 90° C. Evaporate 100 cc of wine in a porcelain dish on the water bath to about 10 cc and treat the residue with about 5 grams of fine sand and with from 3 to 4 cc of milk of lime (containing about 15 per cent of calcium oxid) for each gram of extract present, and evaporate almost to dryness with frequent stirring (avoid formation of dry crust or evaporation to complete dryness). Treat the moist residue with 50 cc of 90 per cent alcohol by volume, wash down the sides of the dish, using a spatula, if necessary, to remove the substance adhering to the sides, and rub the whole mass to a paste. Heat the mixture on the water bath, with constant stirring, to incipient boiling and decant the liquid through a 12.5 cm fluted filter. Wash the residue repeatedly with small portions of hot 90 per cent alcohol, twice by decantation, then transfer all the material to the filter and continue washing until the filtrate amounts to about 150 cc. Transfer the filtrate to a porcelain dish and evaporate to a sirupy consistency on the water bath. Add 10 cc of absolute alcohol to dissolve the residue and transfer same to a 50 cc g. s. cylinder, using two additional portions of 5 cc each of absolute alcohol to wash out the dish and complete the transfer. Add three portions of 10 cc each of absolute ether, thoroughly

[a] U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 12.

[b] U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, p 83, (a) In dry wines.

shaking after each addition. Let stand until clear, then pour off through a filter, and wash the cylinder and filter with a mixture of one part of absolute alcohol to one and one-half parts of absolute ether, pouring the wash liquor also through the filter. Evaporate the filtrate to about 5 cc on the water bath, add 20 cc of water (a marked turbidity appears on the addition of water, which, as mentioned by Hortvet, is due to water insoluble impurities), and evaporate to about 5 cc; again add 20 cc of water and evaporate to about 5 cc; finally add 10 cc of water and evaporate to about 5 cc. (These evaporations remove all the ether and alcohol without loss of glycerin, since by keeping a minimum volume of 5 cc the concentration is not greater than 20 per cent and there is little danger of loss of glycerin at 90° C. up to 50 per cent concentration.) Transfer the residue with hot water to a 100 cc g. s. volumetric flask, cool, add silver carbonate freshly precipitated from 0.1 gram silver sulphate, shake and allow to stand ten minutes; then add 0.5 cc of lead subacetate solution, shake occasionally and allow to stand ten minutes; make up to the mark, shake well, filter, rejecting the first portion of the filtrate, and introduce 25 cc of the clear filtrate (measured from a 25 cc burette) into a 250 cc g. s. volumetric flask. Add 1 cc of concentrated sulphuric acid to precipitate the excess of lead. (Unless a small quantity of sulphuric acid is introduced prior to the addition of the bichromate, the latter will combine with the excess of lead in solution and precipitate lead chromate, thereby causing a slight error.) The purified glycerin solution is now ready for oxidation by the following method:

DETERMINATION OF GLYCERIN BY A MODIFIED HEHNER'S BICHROMATE OXIDATION METHOD.[a]

(a) *Solutions required.*

(1) *Strong bichromate:* Dissolve 74.615 grams dry, recrystallized potassium bichromate in water, add 150 cc concentrated sulphuric acid, cool, make up to 1000 cc, at 20° C., and determine the specific gravity of the solution at 20°/20° C.; 1 cc of this solution equals 0.01 gram glycerin. The high coefficient of expansion of this strong solution makes accurate volumetric measurement difficult on account of the changes in room temperature from day to day and it is therefore advisable to take the specific gravity and use weighed amounts of the solution, which may be conveniently introduced into the tests by means of a weight burette. Then the weight of the solution used in a given test divided by the specific gravity equals the volume used. The strong bichromate solution has an apparent expansion in glass of 0.0005 (or 0.05 per cent) for each degree centigrade. By observing this correction the solution may be measured in the absence of a weight burette.

(2) *Dilute bichromate:* Introduce a weighed amount (12.5 times the specific gravity) of the strong bichromate from a weight burette into a 250 cc g. s. volumetric flask, dilute with water and make up to the mark at room temperature; 20 cc of this solution is equivalent to 1 cc of the strong bichromate. If the weight taken is slightly in excess of the 12.5 cc equivalent, make up to the mark and then add required amount of water to make one-twentieth dilution.

(3) *Ferrous ammonium sulphate:* Dissolve 30 grams of crystallized ferrous ammonium sulphate in water, add 50 cc of concentrated sulphuric acid, cool and dilute to 1,000 cc at room temperature; 1 cc of this solution is approximately equivalent to 1 cc of the dilute bichromate. Its value changes slightly from day to day and should be standardized against the dilute bichromate whenever used.

(b) *Oxidation of glycerin.*

From a weight burette introduce into the 250 cc flask, containing the 25 cc purified glycerin solution, a weighed amount of the strong bichromate solution, sufficient to leave about 12.5 cc excess (with ordinary wines purified as above use 30 to 35 cc), carefully add 24 cc of concentrated sulphuric acid, rotating flask gently to mix contents and avoid violent ebullition, then place in *boiling* water bath for exactly 20 minutes. Remove flask from bath, dilute at once, cool, and make up to mark at room temperature. A slightly more accurate oxidation may be obtained by adding only 15 cc of concentrated sulphuric acid and continuing the digestion for at least 2 hours in a boiling water bath.

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[a] Richardson and Jaffé, J. Soc. Chem. Ind., 1898, **17**: 330, modified by W. H. Low.

(c) *Titration.*

(1) Standardize the ferrous ammonium sulphate solution against the dilute bichromate solution by introducing from the respective burettes approximately 20 cc of each of the two solutions into a beaker containing 100 cc of distilled water. Complete the titration using potassium *ferricyanid* solution as the indicator on a porcelain spot plate. From this titration calculate the volume (F) of ferrous ammonium sulphate equivalent to 20 cc of the dilute and also, therefore, to 1 cc of the strong bichromate solution.

(2) In place of the dilute bichromate solution now substitute a burette containing the oxidized glycerin with excess bichromate solution, and ascertain how many cubic centimeters of it are equivalent to (F) cubic centimeters of the ferrous ammonium sulphate solution, and also, therefore, to 1 cc of the strong bichromate. Then 250 divided by this last equivalent equals the number of cubic centimeters excess of the strong bichromate solution present in the 250 cc flask after oxidation of the glycerin.

(3) The number of cubic centimeters of strong bichromate added minus the excess found after oxidation multiplied by 0.01 gram equals the weight of glycerin in the 25 cc of purified solution used in the determination; this result multiplied by four gives the weight of glycerin in grams per 100 cc of the wine.

The c. p. glycerin used in the work was shown by a careful specific gravity determination to contain 95.25 per cent of glycerol. A 1 per cent glycerin solution was made by using 10.4987 grams of this c. p. glycerin per liter. Two direct bichromate determinations on 25 cc portions of this solution gave 0.9969 and 0.9975 per cent instead of 1.0 per cent.

An imitation dry wine was then made to contain 1 per cent of glycerin, and in addition 1 gram of Rochelle salts, 2 cc of acetic acid, 2.5 grams of commercial glucose, and 0.8 gram of tannic acid per liter. A determination by the above modified provisional method and subsequent oxidation gave 0.9565 per cent glycerin. A determination carried out simultaneously, but dried for one hour at the temperature of boiling water, as in the regular provisional method, gave on oxidation 0.9469 per cent glycerin, showing loss on drying. Three complete determinations were then made on separate 100 cc portions of the imitiation wine, using the new modified method, and the following results were obtained on oxidation: 0.9534, 0.9573, and 0.9588; average, 0.9565 per cent, which is practically 96 per cent recovery of the glycerin present.

A determination similarly made, with the exception that all evaporations were made rapidly on a water bath at a temperature of 100° C. instead of being limited to 90° C., gave only 0.8857 per cent, which would indicate that the temperature control is quite important. This experimental work will be continued, using various known imitation wines and unknown genuine wines.

REPORT ON BEER.

By H. E. Barnard, *Associate Referee.*

The referee on beer has nothing new to report on the analysis of normal beers except the addition to the methods for determining sugars of several formulas which have been worked out primarily for the purpose of determining the character of the brewing material. These formulas have not been tested outside of the referee's laboratory but have there been used very successfully for some time. It is well known that in the brewing of the modern beer barley malt has been largely replaced by substitutes of varying composition and character. Among the best known substitutes are the different forms of glucose, brewer's sugar, anhydrous sugars, rice, corn grits, etc. Many beers in which the barley malt has been largely replaced by such substitutes are still

sold as malt beers. In view of the fact that standards have been set for beers which establish the character of the raw material that may be used in brewing the various forms of beer, it is of importance to be able to determine by analysis what forms of sugars are used by the brewmaster. Attempts have been made to detect the use of glucose and rice by determining the amounts of protein and phosphoric acid present, on the theory that true malt beers are rich in these constituents, while the substitutes are correspondingly poor. This method of differentiation was first described by Parsons,[a] and works very well on beers which are entirely or very largely glucose or rice products, but fails ignominiously to detect the use of less than 50 per cent of glucose in a pure malt beer. This is due to the fact that barley malt itself varies so widely in its nitrogen and phosphoric acid content that the shrewd brewer by choosing the proper malts may produce a beer from a 50 per cent glucose wort that is higher in these constituents than another all-malt beer which may have been made from a malt low in these materials. The sugars present in a genuine malt beer are chiefly maltose and the unfermentable starch dextrin, and the beer contains very little, if any, dextrose. The glucose sugars, on the contrary, are low in maltose and high in dextrose. It is evident then that a beer low in maltose and high in dextrin and dextrose is a glucose beer, and, on the contrary, a beer high in maltose and low in dextrose is probably a malt beer. So by taking also into consideration the nitrogen, phosphoric acid, and extract contents, it is possible to determine with a fair degree of accuracy the types of material used in brewing a part malt beer. The analytical difficulties heretofore met with in the estimation of the dextrin, dextrose, and maltose content of beers have been so serious that the separation of the sugars has not usually been attempted. Therefore, some work was done along this line which, while perhaps not entirely new, has never before been attempted in exactly the same way.

W. D. McAbee, assistant in this laboratory, who has given much time to beer analysis during the last two years, has originated several formulas which simplify these determinations and, so far as they have been tested, give accurate results. Various formulas have been derived for the determination of dextrin, dextrose, and maltose when in one solution, but they involve complicated methods, and are, for the most part, unsatisfactory, especially so in the analysis of beer, because of the small amounts of sugars present.

These formulas are derived from equations deduced from the amount of copper reduced by each of the sugars and from the rotatory powers of dextrin, dextrose, and maltose. It is a well-known fact that a solution of dextrin containing 1 gram in 100 cc will polarize 11.2° (sugar scale), a solution of dextrose of the same strength 3.05°, and maltose 8.1°. It has also been proved that 1 gram of dextrose will reduce 2.2 grams of cuprous oxid, while 1 gram of maltose will reduce 1.3 grams.

If the dextrin in the beer is removed by precipitation with alcohol, the polarization taken on the filtrate and calculated to the original volume will be due either to dextrose, maltose, or both. The difference between this reading and the original polarization will be due to dextrin, and therefore the grams of dextrin in 100 cc of beer may be calculated as follows:

$$\text{Dextrin} = \frac{P - p}{11.2},$$

where P is the original polarization and p the polarization due to sugars.

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[a] J. Amer. Chem. Soc., 1902, **24**: 1170.

Since the polarization after the removal of dextrin is due to the sugars, the following equation may be found from the rotatory powers of dextrose and maltose:

$$(1)\ 3.05\ D+8.1\ M=p,$$

where D equals the grams of dextrose and M the grams of maltose in 100 cc. Similarly, since the reducing powers of dextrose and maltose are known, the following equation may be derived:

$$(2)\ 2.2\ D+1.3\ M=C,$$

in which D equals the grams of dextrose per 100 cc, M the grams of maltose per 100 cc, and C the grams of cuprous oxid reduced by 100 cc.

Equations (1) and (2) are simultaneous, having the same unknown quantities, N and M, and can be solved algebraically. Solving for D and M gives the following formulas:

$$D=\frac{6.23\ C-p}{10.6}$$

$$M=\frac{p-1.38\ C}{6.3},$$

in which D equals grams of dextrose per 100 cc; M grams of maltose per 100 cc; p, polarization due to sugars, and C the cuprous oxid reduced by 100 cc of beer.

The method used to determine P, p, and C is as follows:

Determine P by taking the sugar scale reading of the beer in a 200-mm tube. Evaporate 25 cc of the beer on the water bath to a sirupy consistency in an evaporating dish. Add 25 or 30 cc of absolute alcohol, stirring while adding. Let stand for an hour, decant the supernatant liquid to a 50-cc flask, wash the dextrin in the dish with more alcohol, and make up the contents of the flask to 50 cc with the wash alcohol. Let stand twelve hours, or until the dextrin has settled out, filter, polarize in a 200-mm tube, and multiply the reading by 2 to obtain p. C, the amount of cuprous oxid reduced by 100 cc of beer is calculated from the cuprous oxid obtained in the usual manner.

There are a few precautions which should be observed in following this method. The most important is the removal of the dextrin. The beer should be evaporated to the consistency of a rather thin sirup, usually about 8 cc; for if it is not evaporated sufficiently the excess water will hold some of the dextrin in solution. Before polarizing the alcoholic filtrate from the dextrin, it is well to test it by adding an excess of alcohol to 2 or 3 cc of the filtrate in a test tube. If the dextrin has been completely removed, no turbidity will appear in the solution. If these directions are followed closely, no trouble should be experienced in completely precipitating the dextrin.

For the benefit of those who are accustomed to weighing the copper as metallic copper, the sugar formulas are revised as follows: If C equals grams of copper reduced by 100 cc of beer, then—

$$\text{Dextrose}=\frac{7.04\ C-p}{10.32}$$

$$\text{Maltose}=\frac{p-1.6\ C}{6.26}.$$

While these formulas were intended originally for the analysis of beer they may be applied to the analysis of commercial glucose products by using a 10 per cent solution.

The sugar content of four well-known beers when compared with the sugar content of a temperance beer admittedly brewed from glucose is well illustrated by the following table:

Sugar content of typical beers. (*Grams per 100 cc.*)

Brand.	Dextrin.	Dextrose.	Maltose.
Standard beers:			
Gold Medal	3.12	0.08	1.54
Budweiser	2.90	.10	1.28
Blue Ribbon	3.19	.12	1.19
Hop Cream	2.43	.00	1.20
Temperance beer:			
Tonica	4.65	1.90	.03

REPORT ON DISTILLED SPIRITS.

By L. M. Tolman, *Associate Referee.*

The work on distilled spirits for this year was limited to testing the quantitative value of the Marsh test, in separating caramel coloring matter from the natural coloring material of whiskies. Five samples, prepared as follows, were sent to each of the collaborators: A sample of genuine 5-year-old whisky, matured in a charred barrel, was taken as the basis; then a 50 per cent, by volume, alcohol solution was prepared and colored with caramel, so as to have exactly the same depth of color as the whisky, and these two solutions were mixed in various proportions, as shown in the table.

Composition of official samples.

Number of sample.	Straight whisky.	Caramel-colored alcohol.
1	All	None.
2	2 parts	1 part.
3	1 part	1 part.
4	1 part	2 parts.
5	None	All.

In sample No. 2, one-third of the coloring matter present was due to the caramel and two-thirds to the natural whisky; No. 3, one-half caramel and one-half straight whisky; No. 4, two-thirds caramel and one-third straight whisky. The method submitted to the collaborators is as follows, and in addition to making the determination as directed, it was requested that the sample be examined without evaporation, as all of the samples sent out were 100° proof:

Evaporate 50 cc of whisky just to dryness on a steam bath in a porcelain evaporating dish. Dissolve the residue in water and alcohol, using 26.3 cc of 95 per cent alcohol, and make up to volume in a 50 cc flask. Transfer 25 cc of this solution to a separatory funnel; add 25 cc of the Marsh reagent and shake not too vigorously, to avoid emulsification. Allow the layers to separate and repeat the shaking twice more. After the layers have separated clearly, run off the lower layer into a 25 cc cylinder, and make up to volume with 50 per cent by volume alcohol. Compare in a colorimeter with the remaining 25 cc portion (which has not been extracted with the reagent) and express the results as per cent of color insoluble in amyl alcohol.

Marsh reagent.—Mix 100 cc of amyl alcohol, 3 cc of sirupy phosphoric acid, and 3 cc of water; shake before using. If the reagent becomes colored on standing, the amyl alcohol should be redistilled over 5 per cent phosphoric acid. Fourteen sets of samples were sent out and reports have been received from seven. The following table gives the results of the cooperative work:

Percentage of color in official samples insoluble in amyl alcohol.

Collaborators.	No. 1.		No. 2.		No. 3.		No. 4.		No. 5.	
	Direct.	After evaporation.	Direct.	After evaporation.	Direct.	After evaporation.	Direct.	After evaporation.	Direct.	After evaporation.
T. F. Pappe	2.8	3.0	25.2	24.7	39.2	41.2	47.8	47.8	69.2	66.9
R. W. Hilts	4.5	4.5	27.5	27.5	37.5	39.0	52.5	52.0	67.0	70.0
W. C. Burnett [a]	8.0	8.0	28.0	28.0	40.0	40.0	48.0	50.8	72.6	73.0
J. P. Street [b]	.....	.....	28.4	28.1	40.5	36.7	53.0	55.8	75.0	75.2
H. H. Hanson [c]	0.5	0.8	27.0	32.0	40.0	44.0	56.5	60.0	89.8	90.0
	[d] 6.0	.....	[d] 29.0	.....	[d] 35.0	.....	[d] 55.0	.....	[d] 74.0	.....
J. M. Bartlett [c]	0.2	1.0	30.0	33.0	40.0	45.0	57.0	65.0	90.0	90.0
	[d] 6.0	.....	[d] 23.0	.....	[d] 33.0	.....	[d] 51.0	.....	[d] 80.0	.....
C. R. Smith	very small.	very small.	25.0	28.0	44.0	45.0	51.0	54.0	82.0	80.0

[a] All figures average of two determinations. (Were reported as color soluble in *amyl alcohol.*)
[b] Used Duboscq colorimeter; all results average of at least five determinations.
[c] Were reported as color soluble in amyl alcohol; results obtained from rough apparatus constructed from test tubes.
[d] Results obtained on second trial.

The comments of the collaborators are as follows:

R. W. Hilts: So far as I can see, the evaporation has hardly any effect on the results, for the variations are probably within the limits of observational error of the method and instrument.

J. M. Bartlett: We do not, however, have a colorimeter, and were obliged to use a rather rough apparatus constructed by ourselves from test tubes. Therefore, our determinations may not be as accurate as you desire.

John Phillips Street: The results were obtained by a Duboscq colorimeter and the figures in the first two columns of the tables represent colorimeter readings in millimeters. To my unpracticed eye the best results were secured by starting with both prisms at zero, and using the lighter shaded, that is, short columns of the original liquor. I have reported all my readings to illustrate this point. In the first set of determinations, after drawing off the lower layer from the separator, I made up to 25 cc with water by mistake instead of using 50 per cent alcohol, and these results are reported as Method 3. Whether alcohol or water was used at this stage seemed to have little practical effect on the results. The method seems to be excellent if too close conclusions as to quantity of color added are not attempted.

Results by Method 3 are as follows: Sample No. 2, 27.4 per cent; No. 3, 36.9; No. 4, 52.3, and No. 5, 75.7.

It will be seen from the table that the various analysts have obtained results which agree closely, showing that the method is satisfactory, and give a very close approximation of the amount of natural color present and the amount of caramel. It is evident that caramel is slightly soluble in amyl alcohol, as only approximately 80 per cent is found to be insoluble. It is also apparent that the natural color of the whisky is almost entirely soluble, only a trace being insoluble.

In connection with these results a chart has been prepared showing the amount of caramel removed by this method. From this chart (fig. 2) it will be seen that the percentage of caramel removed is extremely uniform, and is not affected by the natural coloring matter of the whisky at all. The following table, based on the chart, gives the per cent of color which is insoluble in amyl alcohol and the equivalent per cent of color due to caramel. From either the

table or the chart the approximate amount of color in a whisky due to caramel and the amount due to the natural color of the whisky can be readily obtained.

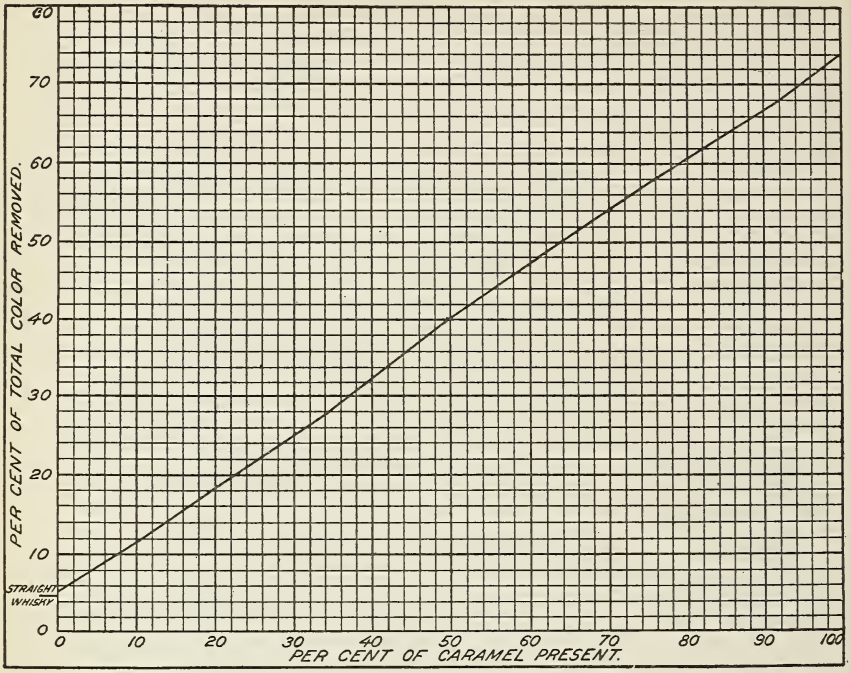

FIG. 2.—Relation between caramel present and amount of color insoluble in amyl alcohol.

This information is extremely valuable in questions of adulteration of whiskies, giving at once the information as to the natural color of the whisky before caramel has been added.

Relation between color insoluble in amyl alcohol and amount due to caramel.

Insoluble in amyl alcohol.	Due to caramel.	Insoluble in amyl alcohol.	Due to caramel.	Insoluble in amyl alcohol.	Due to caramel.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
5.4	0	28	34.5	52	66.5
6	1	30	37.0	54	69.5
8	4.5	32	39.5	56	73
10	6.5	34	42	58	76
12	10.5	36	46.5	60	79.5
14	13.5	38	47	62	82.5
16	16.5	40	49.5	64	85.5
18	19.5	42	52.5	66	88.5
20	22.5	44	55.5	68	92
22	25.5	46	58	70	95
24	28.5	48	61	72	98
26	31.5	50	63.5	74	100

REPORT ON VINEGAR.

By R. W. Balcom, *Associate Referee.*

Compiled Data on Vinegar Analysis.

At the meeting of 1908 no recommendations for future work were made. Owing to this and to the early date of the meeting no cooperative work has been done, but the referee has a few facts to submit which may prove of interest to the association, and these, together with some suggestions and recommendations, form the basis of this report.

It is generally supposed that cider vinegar is characterized by the presence of malic acid, and much time has been spent in trying to find a simple and reliable method for the determination of this acid. Apple juice undoubtedly contains malic acid in varying amounts, but the work of Van Slyke [a] shows that the malic acid content decreases when the juice undergoes fermentation, this action continuing in some cases until the malic acid practically disappears.

Browne [b] makes a similar observation, and W. Mestrezat [c] states that when a must containing malic acid is subjected to fermentation part of the acid disappears, the amount varying with the species of yeast used and also with the nature of the medium, the loss being greater in a richly nutritive medium, such as grape must, than in one poorer in nutritive value.

Van Slyke's work shows further, that in the case of apple juice, the disappearance of the malic acid is most marked just previous to the acetic fermentation, and he states that, as a rule, when the cider has become good vinegar, there remains only a trace of fixed acid, and that in the case of some old vinegars all fixed acid disappears.

There can be no doubt that malic acid is destroyed in some way during the process of fermentation. Just how this takes place and what the decomposition products are may be of interest and worthy of investigation, but one is forced to the conclusion that a quantitative determination of malic acid as such, or even a qualitative test for the same, is of practically no value in forming an opinion as to the genuineness of cider vinegar.

The report of the associate referee last year was almost wholly confined to a discussion of the determination of the lead number of cider vinegars of known purity. The tabulated data given include the values of the lead number for 28 samples of vinegar. These values range from 0.075 to 0.290. It was hoped that limiting values for this quantity might be set, but the range in values is too great to permit of this.

In one case a number as low as 0.056 was found by the referee, and in the examination of several hundred commercial samples no more information as to the genuineness of the product was obtained from the lead number than from noting the amount and character of the precipitate produced by lead acetate in a qualitative test alone. The practice of making a determination of the lead number was therefore discontinued, and the precipitate produced by lead acetate in a qualitative test described as slight, normal, or heavy, as the case might be.

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[a] New York Agr. Exper. Sta., Geneva, Bul. 258.

[b] Ann. Rept., Pennsylvania Dept. Agr., 1901, pp. 128–9; J. Amer. Chem. Soc., 1903, **25**: 24.

[c] J. Soc. Chem. Ind., 1908, **27**: 763; ibid., 1909, **28**: 734.

Early in the year the suggestion was made that it might be of value to determine the nonsugar solids of the vinegar by deducting the total sugar from the total solids, just as is done in the case of milk, where the solids not fat are obtained by deducting the fat from the total milk solids. For the purpose of detecting certain kinds of adulteration this has, perhaps, proved to be of more value than any other single determination.

About 50 letters were sent out to chemists all over the country asking them to report the results of any analyses which they might have made of cider vinegars of known purity. This appeal had no result except to show that besides those already published very few such analyses exist. The writer, however, has tabulated nearly 100 analyses which are more or less complete. These are mainly the analyses published by Smith,[a] Doolittle,[b] Leach and Lythgoe,[c] and by the referee on vinegar in the report of last year.[d] The remainder were furnished by F. C. McCarter. There are others available, but the referee has not as yet been able to obtain them.

It was hoped by getting analyses from different localities to eliminate, in the averages, the variations that might occur in vinegars made from fruit grown in different parts of the country. This has been only partially successful, because, of the analyses used in computing these averages, over two-thirds were of vinegars made from fruit grown in New England. These averages, however, may fairly be said to represent the approximate composition of a cider vinegar which may be taken as a norm for the whole country. When necessary the results have been recalculated to grams per 100 cc unless otherwise stated, and reducing sugars calculated as invert sugar. The average, maximum, and minimum values for some of the more important constituents, and certain relations of these to one another, together with the number of analyses upon which the computation was based, are as follows:

Comparisons of average, maximum, and minimum data compiled from 100 vinegar analyses.

[Grams per 100 cc.]

Determination.	Average.	Maximum.	Minimum.	Number of analyses.
Total acid as acetic	4.94	7.96	3.29	85
Total solids	2.54	4.52	1.37	94
Nonsugar solids	1.90	2.89	[a] 1.26	63
Reducing sugars in solids (per cent)	19.6	45.0	5.6	63
Total ash	0.367	0.52	0.20	81
Alkalinity of water-soluble ash (cc)	35.7	56.0	21.5	65
Ash in non-sugar solids (per cent)	18.8	26.5	11.2	52
Soluble phosphoric acid (P_2O_5)	17.3	39.9	6.7	72
Insoluble phosphoric acid (P_2O_5)	12.0	32.0	4.3	71
Total phosphoric acid (P_2O_5)	29.3	64.2	15.1	71
Polarization (direct) V°	−1.46	−3.6	−0.2	56
Polarization (invert) V°	−1.69	−3.1	±0.0	20

[a] Abnormally low; the next lowest values are 1.35, 1.35, and 1.39. These four are the only ones out of a total of 63 analyses found to be below 1.40.

As was foreseen, owing to the variable amount of sugar present in the total solids, the non-sugar solids prove to be a more constant quantity than the amount of total solids. By reason also of this variable amount of sugar in

[a] J. Amer. Chem. Soc., 1898, **20**: 3.

[b] Report of the Dairy and Food Commission of Michigan, 1904, p. 152.

[c] J. Amer. Chem. Soc., 1904, **26**: 375.

[d] Hickey, U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 29.

the total solids, the percentage of ash in the total solids, a relation which has hitherto been considered to have some value, is made to vary within such wide limits that its usefulness is much impaired if not destroyed altogether. The percentage of ash in the non-sugar solids is much more constant, and may be of service in the detection of adulteration.

Adulteration as Shown by Analytical Data.

How the determination of the non-sugar solids, and to a lesser degree the percentage of ash in the same, helps in the detection of adulteration is shown by the following table, the samples compared having the following character:

No. 1 is the norm established by the 100 compiled analyses with the average, maximum, and minimum values given.

No. 2 is a blend of about 50 commercial samples, the analysis of which showed that they must be passed as pure cider vinegars. The fact that most of the values are below those of the norm indicates that to some of the samples some diluent, probably water, had been added in small amounts, while the relatively high proportion of reducing sugars in the total solids would indicate that to a few, perhaps, some foreign material high in reducing sugar had been added.

No. 3 is an uncolored spirit vinegar.

No. 4 is a mixture of equal volumes of Nos. 2 and 3. The lowering of the total solids and of some of the other results is noticeable, also the reversing of the relation between the phosphoric acid of the water-soluble and the water-insoluble ash, but most marked is the decrease in the amount of the nonsugar solids. It is also to be noticed that the percentage of ash in the nonsugar solids is very high.

No. 5 is a known mixture of cider vinegar, spirit vinegar, and boiled cider. The solids have been brought up by adding the boiled cider. The addition of the boiled cider incidentally also increases to some extent the ash and phosphoric acid. The alkalinity of the ash of course remains normal. There is, however, the same relation between the soluble and insoluble phosphoric acid as in No. 4, and the percentage of ash is abnormally high. Most significant, however, is the low value of the nonsugar solids considered in connection with the abnormally high percentage of sugar in the total solids.

No. 6 is a commercial sample. The similarity between the analyses of Nos. 5 and 6 is very apparent. It may not be out of place at this point to call attention to the fact that an analysis of either No. 5 or No. 6, if made in the ordinary way, would show very little abnormality. The analysis of No. 6 is given because it is typical of a large number of vinegars found on the market at the present time. Beyond doubt these are mixtures of cider vinegar and dilute acetic acid, the latter probably in the form of spirit vinegar, to which has been added some foreign material high in sugar. Whether this material is unfermented apple juice, boiled cider, apple jelly, or some other material is difficult, if not impossible, to say with certainty. In most of these cases it is probably either the unfermented apple juice or boiled cider.

No. 7 is a sugar vinegar, the analysis of which was furnished by Mr. Bisbee, of the United States Food Inspection Laboratory at St. Louis. This vinegar was made by diluting New Orleans molasses to 12° B. and fermenting by adding 60 gallons of brewer's yeast to 2,500 gallons of the diluted molasses. After one day's vigorous fermentation, the product was forced through sand to remove a portion of the yeast, if possible. This filtered product was then mixed with an equal quantity of finished sugar vinegar from the generators and the mixture passed through the generators. This sample is the finished generator product, and the analysis is fairly representative of this kind of vinegar.

Comparison of analyses of vinegars of known characters with commercial samples and with average data.

[Grams per 100 cc.]

Sample No.	Total acid.	Total solids.	Non-sugar solids.	Reducing sugars in solids.	Total ash.	Alkalinity of water-soluble ash.	Ash in non-sugar solids.	Phosphoric acid (P_2O_5).			Polarization (direct).
								Soluble.	Insoluble.	Total.	
				Per ct.		*cc.*	*Per ct.*				*V°.*
1[a]	4.94	2.54	1.90	19.6	0.367	35.7	18.8	17.3	12.0	29.3	−1.46
	7.96	4.52	2.89	45.0	0.52	56.0	26.5	39.9	32.0	64.2	−3.6
	3.29	1.37	1.26	5.6	0.20	21.5	11.2	6.7	4.3	15.1	−0.2
2	4.65	2.40	1.51	37.2	0.32	31.9	21.2	12.4	10.5	22.9	−1.3
3	4.31	0.18	0.16	11.1	0.016	1.5	10.0	0.2	1.5	1.7	+0.6
4	4.51	1.27	0.80	37.0	0.20	17.5	25.0	5.5	7.4	12.9	−0.3
5	4.72	2.15	1.05	51.2	0.28	37.0	26.7	8.7	10.4	19.1	.....
6	4.46	2.11	0.91	56.9	0.29	33.0	31.9	11.5	10.8	22.3	−1.0
7	4.66	2.09	1.73	17.0	0.56	11.1	32.4	1.8	15.2	17.0	±0.0

[a] Average, maximum, and minimum data on about 100 vinegar samples.

The practice of making vinegar from products of the sugar cane to supply the demand for a cheap colored vinegar, which was formerly filled by the sale of artificially colored spirit vinegar, has grown up almost entirely within the last two years. The material used may be a very cheap grade of cane sugar, or low-grade molasses, or what is known as "cistern bottoms," the product deposited when cane juice is stored in cisterns for any length of time. In addition, a vinegar is made from the two commercial varieties of grape sugar. This has practically all the properties of a glucose vinegar, although the manufacturers object to the use of the latter term and prefer to call this product sugar vinegar. It has been possible to obtain only a very limited number of analyses of sugar vinegars, and more analytical data are needed.

The analysis of the sugar vinegar is given for the purpose of showing how closely it resembles that of a cider vinegar. The most notable differences are the high ash, low alkalinity of the water-soluble ash, and very low water-soluble phosphoric acid. The polarization in this particular case is zero. In the three or four analyses which have been tabulated, the polarization is the same before and after inversion. When raw sugar is used the polarization appears to be minus and of about the same value as for a cider vinegar. When molasses is used, the polarization is likely to be plus, probably owing to the gums and other impurities of that nature present. Analytical data are, however, at present too meager to warrant a positive statement on this point.

With increasing refinement in the methods used for the adulteration of vinegar it is evident that the purity of a given sample can not as a rule be based on any one determination. A more or less complete analysis is necessary.

Recommendations.

As the purpose of this association is to ascertain and establish the best available methods of analysis, attention is called to some of the methods in use for the analysis of vinegar.

Karl Windisch[a] states that the ordinary direct determination of total solids in vinegar gives too high a value owing to the incomplete volatilization of the acetic acid. He recommends the addition of water and a second evaporation to dryness on the water bath before drying in the oven for two and one-half

[a] Deut. Essigindustrie, 1908, **12**: 257; Chem. Abs., 1909, **3**: 810.

hours. The referee has done some work to test the truth of this assertion and agrees with Windisch. Where the amount of total solids is small and the determination is made on 10 cc of the vinegar, the error introduced by the incomplete volatilization of the acid is not very large, but when the amount of total solids is large it may amount to 0.1 per cent or more.

It has also been asserted that when a cider vinegar is subjected to steam distillation the distillate is often found to have the power of reducing Fehling solution, owing to the presence of some volatile substance with reducing properties, and the accuracy of the determination of the reducing sugars in a vinegar is thereby affected, in some cases very appreciably.

The referee believes that the method for the determination of reducing sugar in vinegar can be simplified to advantage by omitting the clarification with lead acetate and making the determination directly upon the filtered vinegar. All of these questions should be made the object of cooperative work during the coming year.

It is recommended that—

(1) The question of the influence of the nonvolatilization of acetic acid on the determination of the total solids in vinegars be further investigated.

(2) The question of methods for the determination of reducing sugars in vinegar be further studied.

(3) Paragraph 2, Calculation of Results (Bul. 107, Rev., p. 102), of the present provisional methods be changed to read as follows: "Express all results as grams per 100 cc of vinegar except when otherwise directed. If per cent by weight is desired, calculate from the specific gravity.

(4) Paragraph 3, Specific Gravity, be changed to read as follows: "Determine at 20° C. by means of a pyknometer, a small accurately graduated hydrometer, or a Westphal plummet on the analytical balance. Express as specific gravity 20° C./4° C."

(5) In Paragraph 8, Phosphoric Acid of the Ash, the following change be made: Substitute for "Express the results as milligrams of phosphoric acid in 100 grams of vinegar" the following: "Express the results as milligrams of phosphoric acid (P_2O_5) in 100 cc of vinegar."

The reason for the changes in paragraph 2 are the following: Vinegar is usually measured rather than weighed in making an analysis, because it is a more convenient as well as a shorter proceeding. This being the case, there is no reason why the results of the analysis should not be expressed in grams per 100 cc just as is done in the case of wines, etc. Moreover, the standards for vinegar are expressed in grams per 100 cc, and the lack of agreement now existing is undesirable.

The reasons for the recommendation of the change in paragraph 3 need not be discussed. This recommendation is in line with the general movement to adopt a uniform temperature of 20° C. for this and similar determinations. The change in paragraph 8 follows as a result of the change in paragraph 2.

REPORT ON FLAVORING EXTRACTS.

By E. M. Chace, *Associate Referee.*

Vanilla Extracts.

OUTLINE OF WORK AND COOPERATIVE RESULTS.

The work upon vanilla extracts was outlined, (1) with a view to proposing for official adoption, the provisional method for vanillin and coumarin; (2) to test the colorimetric method for the determination of vanillin for the purpose of its improvement or elimination from the provisional methods; (3) to obtain data upon which to base new and more accurate methods for the

detection of caramel, prune juice, and other artificial coloring matters; and, (4) to ascertain the effect of glycerin on the precipitation of vanilla resins.

It is highly desirable that more satisfactory methods should be obtained, especially along the line of quantitative or semiquantitative determinations of vanilla resins and for the detection of artificial coloring matter, prune juice, and sherry residues. Several investigators are now turning their attention toward vanilla extracts, and it is hoped that within the course of another year the way will have been cleared for a general improvement of the methods of analysis.

Four samples of vanilla extracts were sent out to some sixteen collaborators [a] with the following instructions: Determine vanillin; (a) gravimetrically, (b) colorimetrically; coumarin and acetanilid, according to the provisional methods as given in Bulletin No. 107. Test for coloring matters and for glycerin, giving in detail confirmatory test.

The samples were as follows:

No. 1 was an extract prepared by U. S. P. methods from a medium grade of vanilla beans.

No. 2 was prepared by solution of a well-known brand of oleoresin of vanilla in alcohol using the amount directed upon the label of the product, the color being brought up by a mixture of anilin dyes.

No. 3 was an extract prepared by U. S. P. methods but, in addition, contained both glycerin and caramel.

No. 4 was a purely factitious product compounded of prune juice, sugar, and a special vanilla color made by extracting vanilla beans with hot dilute ammonia evaporated to dryness and taken up in water, and contained 0.1 per cent of vanillin, 0.08 per cent of coumarin, and 0.05 per cent of acetanilid.

The reports on these constituents as received from collaborators are given in detail in Table 1.

TABLE 1.—*Results on vanillin, coumarin, and acetanilid.*

Analysts.	Vanillin.				Sample 4.	
	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Coumarin.	Acetanilid.
E. R. Lyman	0.28	0.11	0.27	0.12	0.06	0.05
A. P. Sy	.15	.06	.16	.06	.06	.06
H. M. Loomis	.22	.08	.22	(a)	(a)	(a)
A. S. Mitchell	.22	.06	.21	.10	.03	.07
R. W. Hilts	.22	.08	.23	.12	.07	.03
W. A. Bender	.17	.06	.23	.14	.11	.04
R. S. Hiltner	.19	.06	.19	.10	(a)	(a)
C. O. Dodge	.22	(a)	.23	.11	(a)	(a)
C. P. Wilson	.20	.07	.21	.11	.05	.02
Maximum	.28	.11	.27	.14	.11	.07
Average	.21	.07	.24	.11	.06	.045
Minimum	.15	.06	.16	.06	.03	.02

[a] Not reported.

COMMENTS BY COLLABORATORS.

E. R. Lyman reported the absence of glycerin as inferred from the hard character of the solids residue. The presence of glycerin in sample No. 4 was unintentional, being present in the prune juice which was used in making up

[a] C. O. Dodge, Bureau of Chemistry, prepared all samples and sent out the preliminary notices to the collaborating chemists.

the sample. In samples Nos. 1 and 3 this collaborator reported that the color was but partially removed by fuller's earth and that Marsh's test for caramel showed no concentration of color in the water layer, thus failing to find the small amount of caramel contained in sample No. 3. He also reported that the acetanilid responded to the U. S. P. test with bleaching powder and phenol, but that the provisional Ritsert's tests proved unsatisfactory both on the acetanilid in the sample and on the pure reagent.

A. P. Sy reported that he followed the directions in Bulletin No. 107 for the samples sent, but that the following modification gave excellent results: Weigh 50 grams into a 400 cc beaker, dealcoholize as directed in the provisional method, add lead acetate, make up to 100 cc, mix, and allow to settle. Decant 50 cc through a filter and proceed with the extraction as directed in the provisional method.

Residues were obtained upon all samples in determining coumarin, but it was reported only after qualitative identification. This analyst also reported the failure of Ritsert's tests when only small quantities of acetanilid were present.

H. M. Loomis reported the following work on the detection of coloring matter:

TABLE 2.—*Detection of coloring matter in official samples.*

Test.	No. 1.	No. 2.	No. 3.	No. 4.
Resins	Indicate color from vanilla bean.	Indicate small amount of color from vanilla bean.	Indicate color from vanilla bean.	Indicate absence of vanilla bean color.
Double dyeing on wool.	No coal-tar dye.	Coal-tar dye present.	No coal-tar dye.	No coal-tar dye.
Marsh color test; color in amyl alcohol layer.	75 per cent—indicates vanilla bean color.	70 per cent	65 per cent—indicates vanilla bean color.	35 per cent—indicates color foreign to vanilla bean.
Fuller's earth test; color removed.	About 13 per cent.		About 15 per cent.	About 75 per cent—indicates caramel.

EXTRACTION WITH IMMISCIBLE SOLVENTS.

Sample No.	Acetic ether.		Amyl alcohol.		Acetone from color solution; saturated with salt.	
	Neutral.	Acid.	Neutral.	Acid.	Neutral.	Acid.
1	Reagent yellow-brown; H_2O brown.	Reagent brown; H_2O brown.	Reagent orange cloudy; H_2O brown and turbid.	Reagent orange brown; H_2O brown and turbid.	Reagent red-brown; H_2O brownish yellow.	Reagent red-brown; H_2O brownish yellow.
2	Reagent dirty yellow.	Reagent yellow; H_2O orange.	Reagent orange; H_2O yellowish.	Reagent orange; H_2O pinkish.	Reagent yellow; H_2O orange.	Reagent orange; H_2O orange.
3	Reagent brownish yellow; H_2O brown and cloudy.		Reagent brownish yellow; H_2O b.own.	Reagent brownish yellow; H_2O brown.	Reagent brown; H_2O yellow-brown.	Reagent red-brown; H_2O yellowish brown.
4	Reagent nearly colorless; H_2O pale yellow.	Both nearly colorless.	Both nearly colorless.		Both nearly colorless.	Reagent brown; H_2O yellow-brown.

This analyst also determined vanilla resins as follows:

Slightly acidify 50 cc of extract with acetic acid, free from alcohol by evaporating on water bath to 25 cc, make up to 50 cc with water, and evaporate again to 25 cc. Dilute to 50 cc, cool, filter through a wet filter, and wash

thoroughly with cold water till free from water-soluble substances. Then dry the filter and precipitate and extract with 95 per cent alcohol, collect the extract in a tared dish, evaporate, dry, and weigh as resins.

The phenylhydrazin test for caramel as modified by Woodman, showed that caramel was present in No. 4 but absent in Nos. 1 and 3, failing to detect the small amount present in the latter.

W. A. Bender reported that in extracts Nos. 1 and 2, where the vanillin residue looked very impure, they were first weighed, then extracted repeatedly with a mixture of petroleum and ether in the proportion of three parts of the former to two parts of the latter. Residues were then dried, weighed, and subtracted from the first weight, giving the weight of pure vanillin. Ritsert's tests for acetanilid as given in the provisional methods were again reported as unsatisfactory.

R. W. Hilts, in commenting on the test for caramel as described by Woodman, states that he uses 15 cc of extract in a Hortvet tube, 2 cc of zinc chlorid solution (5 per cent) thoroughly mixed with 2 cc of potassium hydroxid (2 per cent solution). The mixture is placed in a centrifuge tube, swung, and the supernatant liquid decanted. The precipitate is then washed twice with 15 cc of water, centrifuging and decanting, a little salt being added to the water in order to aid the settling. The precipitate is then taken up in acetic acid as described by Woodman, evaporated to a small volume, nearly neutralized, and filtered into a test tube. It is then precipitated with paraldehyde, 3 volumes, and allowed to stand overnight. His experience, however, does not agree with that of Woodman that a brown flocculent precipitate always indicates caramel. Such precipitates have been obtained in small amounts for authentic U. S. P. extracts. Caramel, however, on standing overnight gives an adherent deposit, while precipitates from genuine extracts remain flocculent and adhere to the walls of the tube. This analyst reports no success with the phenylhydrazin test of Woodman. For confirmatory tests for natural color he prefers extraction with one volume of ether.

Three analysts have reported results on vanillin obtained by the colorimetric methods as shown in table No. 3.

TABLE 3.—*Results by the colorimetric method for vanillin.*

Analyst.	Sample No. 1.		Sample No. 2.		Sample No. 3.		Sample No. 4.	
	Gravimetric.	Colorimetric.	Gravimetric.	Colorimetric.	Gravimetric.	Colorimetric.	Gravimetric.	Colorimetric.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
W. A. Bender	0.17	0.13	.....	.....	0.22	0.14	0.10	0.10
H. M. Loomis	.22	.14	0.07	0.07	.22	.14	.....	.07
A. P. Sy	.....	.....	.....	.....	.15	.04	.06	.04

These results are exceedingly unsatisfactory. In all of the cases, with one exception, the result is much lower than that obtained by the same analysts using the gravimetric method. The majority of collaborators commented on the method, the general opinion being that it was very unsatisfactory as set forth in the provisional methods. Following are the comments on the colorimetric method:

Mr. R. W. Hilts: I have not yet succeeded in working the colorimetric method for vanillin with any satisfaction, hence do not report any results. The samples show color so far from the standards in tint that comparison was practically impossible.

Mr. W. A. Bender: Leach's colorimetric method was used in the main as given in the provisional methods; 2 cc of extracts were weighed out in test tubes, and the results are, therefore, in percentage by weight. It seemed necessary to use more bromin on the sample than on the standard in order to get a maximum green color; accordingly, after adding 3 drops of bromin water to both sample and standard and an amount of ferrous sulphate, found by trial to give a maximum color, more bromin water was added to the sample until the maximum color was obtained. Three or 4 drops more were found necessary sometimes, making a total in some cases of 7 drops of bromin water. The coal-tar dye used on extract No. 2 faded upon the addition of bromin water, but left a yellow color which interfered seriously with the readings and made it impossible to apply the method to this extract.

A. P. Sy: I have never been able to get good results with the colorimetric method for vanillin. The tints produced by the standards and the samples are seldom sufficiently alike to admit of a good comparison.

E. R. Lyman: The colorimetric estimation of vanillin was found impracticable; the greenish tinge from the extracts and pure vanillin being of a different order, could not be compared with any degree of certainty. Mr. Knisely also tried the method with the same result.

C. D. Howard: I also believe that the direction in connection with the colorimetric method should be made more specific. A great deal depends upon the strength and the way in which the bromin water and ferrous sulphate solution are added, as to depth and character of tint produced. I find that the inexpert worker is liable to encounter a great deal of difficulty in securing accurate results by this procedure as at present laid down. In connection with the use of Schreiner's colorimeter, we have found that comparisons of this color can be more readily effected by artificial light.

Messrs. C. O. Dodge and C. P. Wilson also reported that the tints obtained for genuine extracts were so different from those obtained for the standard as to render comparisons inaccurate, so that no results by this method were reported by them.

The associate referee has not recommended that the colorimetric method be dropped as a provisional method, in the hope that since the matter has received the attention of a considerable number of collaborators, modifications will be suggested and the method so improved as to render it useful in the hands of those who have had only a limited experience with it. No further recommendation is made on the methods for vanillas other than that the work be continued along similar lines.

Lemon Extracts.

INSTRUCTIONS AND RESULTS OF COOPERATIVE WORK.

Four lemon extract samples were sent to the sixteen collaborators and results have been received from eleven. Work was outlined (1) with the intention of recommending the provisional method for citral for official adoption; (2) to test a new colorimetric method using metaphenylene diamin hydrochlorid as a reagent, devised by R. S. Hiltner of the Denver Food and Drug Laboratory, U. S. Department of Agriculture; and (3) to discover the effect of the presence of cane sugar on determinations by these two methods. In preparing the samples and citral standard this year, the citral used was from a lot specially manufactured for the Bureau of Chemistry. The product was chemically pure and derived from lemon oil instead of lemon grass, as is usual.

Four samples were sent out as follows:

No. 5. A solution of citral in 50 per cent alcohol, the finished product containing 0.1 per cent of the aldehyde.

No. 6. A solution of citral in 50 per cent alcohol which had been saturated with cane sugar, the final product containing 0.1 per cent of the aldehyde.

No. 7. An extract prepared from synthetic lemon oil made in the laboratory as follows: Lemon oil from which the citral had been removed by shaking

with bisulphite of soda was treated with a small amount of sodium carbonate until only traces of citral remained. A known quantity of citral was then added to the product, and the final extract was prepared by dissolving it in 90 per cent alcohol in the ratio of 5 grams of the oil to 95 grams of alcohol. The finished extract contained 0.27 per cent of citral.

No. 8. A terpeneless extract of lemon prepared by shaking out the synthetic oil of lemon, described under No. 7, seven times with portions of 250 cc each of 50 per cent by volume alcohol.

The instructions sent with the samples requested that the citral be determined by the provisional method and by Hiltner's method. Directions for the latter were inclosed as follows:

Hiltner's Method for the Determination of Citral.

Reagents.

Metaphenylene diamin hydrochlorid solution.—Prepare a 1 per cent solution of metaphenylene diamin hydrochlorid in 50 per cent ethyl alcohol. Decolorize by shaking with fuller's earth or animal charcoal and filter through a double filter. The solution should be bright and clear, free from suspended matter, and practically colorless. It is well to prepare only enough solution for the day's work, as it darkens on standing.

Alcohol.—For the analysis of lemon extracts, 90 to 95 per cent alcohol should be used, but for terpeneless extracts alcohol of 40 to 50 per cent strength is sufficient. Filter to remove any suspended matter. The alcohol need not be purified from aldehyde. If not practically colorless, render slightly alkaline with sodium hydroxid and distil.

Apparatus.

Any convenient form of colorimeter may be used.

Manipulation.

All of the operations may be carried on at room temperature. Weigh into a 50 cc graduated flask 25 grams of the extract and make up to the mark with alcohol (90–95 per cent). Stopper the flask and mix the contents thoroughly. Pipette into the colorimeter tube 2 cc of this solution, add 10 cc of metaphenylene diamin hydrochlorid reagent, and complete the volume to 50 cc (or other standard volume) with alcohol. Compare at once the color with that of the standard which should be prepared at the same time, using 2 cc of standard citral solution and 10 cc of the phenylene diamin reagent, and making up to standard volume with alcohol. From the result of this first determination calculate the amount of standard citral solution that should be used in order to give approximately the same citral strength of the sample under examination, then repeat the determination.

The results received are given in Tables 4 and 5.

TABLE 4.—*Citral determinations by the provisional method.*

Analysts.	Sample No. 5.	Sample No. 6.	Sample No. 7.	Sample No. 8.	Analysts.	Sample No. 5.	Sample No. 6.	Sample No. 7.	Sample No. 8.
C. O. Dodge	0.10	0.10	0.30	0.19	W. A. Bender	0.10	0.09	0.28	0.18
C. P. Wilson	.10	.09	.28	.16	H. L. Jackson	.11	.09	.27	.17
E. R. Lyman	.10	.11	[a].22	[a].14	R. S. Hiltner	[a].07	.09	.28	.18
W. C. Burnet	.10	.09	.26	.20	A. V. H. Mory	.11	.08	[a].33	.19
F. G. Smith	.08	.08	.25	.18	Maximum	.12	.11	.33	.20
C. D. Howard	.12	.10	[a].20	.19	Average	.103	.092	.280	.180
R. W. Hilts	.11	.09	.30	.19	Minimum	.08	.08	.25	.16
A. P. Sy	.10	.09	.30	.16	Amount present	.10	.10	.27	(?)

[a] Omitted from averages.

TABLE 5.—*Citral determinations by Hiltner's method.*

Analysts.	Sample No. 5.	Sample No. 6.	Sample No. 7.	Sample No. 8.	Analysts.	Sample No. 5.	Sample No. 6.	Sample No. 7.	Sample No. 8.
C. O. Dodge	0.10	0.12	0.28	0.19	H. L. Jackson	0.09	0.11	a.21	0.17
E. R. Lyman	.09	.12	.27	.17	R. S. Hiltner	.11	.13	.26	.18
W. C. Burnet	.10	.15	.27	.19	A. V. H. Mory	.10	.14	a.33	.19
F. G. Smith	.08	.14	.29	.19	Maximum	.12	.15	.29	.21
C. D. Howard	.12	.15	.26	.21	Average	.100	.135	.276	.184
R. W. Hilts	.10	.14	.29	.17	Minimum	.08	.11	.26	.17
A. P. Sy	.11	.15	a.20	a.15	Amount present	.10	.10	.27	(?)
W. A. Bender	.10	.13	.29	.18					

a Omitted from averages.

COMMENTS OF COLLABORATORS.

E. R. Lyman reports that in samples Nos. 5 and 6 the color of the extract interefered to some extent with the determinations by the provisional method. When the color was removed by means of wool and acid, citral was almost entirely removed or destroyed. This collaborator did considerable work upon the error caused by measuring and weighing the extract, and found that it would require approximately 10 times the maximum error of pipetting to affect results of the determination.

W. C. Burnet reports considerable difficulty with the provisional method on sample No. 6, which contained cane sugar, and the results obtained by him in the preliminary examination were much too high, but on reexamining a second sample the result given in the table was obtained. The color developed, however, was so different from the standard in both cases that he reported the results as unsatisfactory.

A. S. Mitchell, reporting the analysis of F. G. Smith, states:

The only variation from the customary method of working was that the nesslerizing was performed in 50 cc flasks with narrow necks, after the manner of the Massachusetts State Board of Health in nesslerizing ammonia in water analysis. An advantage is obtained for the reason that there is little chance of contact with the air. A series of standards is prepared and the unknowns are compared directly with the standards, without dilution.

R. W. Hilts reports that the coloring matter in the samples did not interfere with the comparisons in the provisional method, except to a slight degree in sample No. 6, where the tint developed was somewhat different in shade from that of the standard, making comparisons slightly more difficult than usual. Concordant results, however, were obtained when working this sample on different days. Relative to the permanence of the citral standard, this collaborator reports that he checked a standard which had been kept in a refrigerator for five and one-half months and found that it had lost but 3 per cent of the citral contained therein.

H. Lewis Jackson reports having no difficulty in obtaining aldehyde-free alcohol from the commercial 94 per cent upon boiling with metaphenylene diamin hydrochlorid for twenty-four hours. The coloring matter in the extract did not interfere with his comparisons.

Mr. Mory's comments are as follows:

The colorimetric work furnished the chief source of error. I tried several modifications of the apparatus to lessen this error, and finally concluded that the matching of colors was an art, not a science, and that the difficulty lay more in the eye than in the apparatus. Finally, I adopted the plan of making the readings quickly and being guided by first impressions when the eyes were fresh, with the result that much more consistent results were obtained.

I introduced the additional refinement of reversing the outer tubes in reading and taking the average of the two sets of readings, though I would lay no stress on this, only there appeared at times to be a difference here from some causes other than that of a change in the solutions themselves, as shown by repeated reversal of position. Also, a correction was found necessary owing to differences noted in the graduations of the tubes.

I did not more than touch on the matter of the control of temperature, but would like to raise the question of the importance of control of temperature when identical treatment is given both sample and standard, and more particularly in the final comparison when sample and standard have been brought into close agreement. This has a direct bearing upon the colorimeter work since while prompt readings may be very desirable, hasty readings may easily lead to error. It became very plain after running a number of batches, that I could well afford to neglect any error that might be due to the amount of warming up that a careful reading of the colorimeter made necessary. In the final comparisons, when sample and standard gave nearly equal readings, it was found that the colorimeter readings could be continued until the solution had warmed up very considerably, say to 25° or 30°, without a change in the relative intensities of sample and standard being capable of demonstration by any single set of such readings, although the depth of color was increased in each. In fact, I was not able to demonstrate, beyond the chance that the error found was not that due to the reading of the colorimeter, that there was any change in relative depth of color even on standing overnight at room temperature, though of course in this case the colors had become so deep that it was necessary to read a very small depth of the solution. This is not so true of the readings given by the blank and its standard, for while not showing any change at first, a very decided change was found on long standing. On standing overnight a blank of 1.05 mg changed to 0.35 mg. (See method of calculation given below.)

It would appear to be not unreasonable to expect a sample and its standard when in close agreement to develop color at an equal rate, since there are presumably equal quantities of the two forms of aldehyde present in each. Whether or not there are likely to be found in certain samples other substances that may either develop color on their own account or exert an accelerating or retarding effect on long standing, which difficulties are not encountered at the temperatures and during the time prescribed by the method is a point calling for investigation.

As a corollary to this it would appear to be desirable in the preliminary examination to remove from the bath at the same time all samples which are to be compared with a given standard, for plainly, if in the further development of color the original ratio of sample to standard is not preserved, even when time and temperature are equal, much less may we expect a constant relation if sample and standard are permitted to develop at different temperatures.

Also, I adopted the plan in the final run and when two or more samples were being examined, of fastening the sample and its standard together with a rubber band when first placing them in the bath and then not separating them until they are ready to read, thereby insuring the same starting time for each, the different samples being started about the length of time apart that it required to properly make the colorimeter readings.

It appeared to be more convenient, to weigh approximately 1 gram of the sample each time and vary the standard by measuring, with a small graduated pipette, the quantity of standard citral solution necessary to match the sample. This is not important, and in the case of samples of very low or of very high citral content could not be done, but was followed in the work reported.

I was unable after two trials to prepare, by the method prescribed, alcohol which contained less than the equivalent of about 0.50 mg of citral in the quantity of alcohol used for the test, and this in spite of the fact that the treatment with metaphenylene diamin hydrochlorid was prolonged at each stage. It was necessary, therefore, to adopt a method of calculation which would introduce no theoretical error from this source. This is especially desirable in the preliminary runs when sample and standard are frequently of quite different value. Accordingly, the following formula was used in calculating all results reported:

$$(1)\quad x=\frac{mS+(S-R)b}{R}$$

in which x=milligrams of citral in sample under examination.

b=milligrams of citral equivalent to the quantity of aldehyde in the amount of alcohol used, i. e., in the blank.

m=milligrams of citral added to standard.

S=colorimeter reading given by the standard.

R=colorimeter reading given by the sample.

The value of b was determined by matching the blank against a standard containing a small amount of added citral, say 0.5 mg, b being calculated from the formula:

$$(2)\quad b=\frac{mS}{B-S}$$

in which m and S have the same significance as before and B is the colorimeter reading given by the blank.

Formula 2 may be derived by solving for b in the expression:

$$\frac{S}{B}=\frac{b}{m+b}$$

that is, the reading given by the standard is to the reading given by the blank as the quantity of aldehyde in the blank is to the whole quantity of aldehyde in the standard. This last is plainly made up of the sum of the added citral and the aldehyde present in the amount of alcohol used in making the test, i. e., in the blank.

Formula 1 stated above is given by solving for x in the expression:

$$\frac{S}{R}=\frac{x+b}{m+b}$$

The conditions under which the aldehyde present in the blank is entirely negligible (see formula 1) are that either b or S — R shall be equal to zero. But since b appears seldom, if ever, to be zero, and as there is, particularly in the preliminary test, frequently considerable difference between the readings given by the standard and by the sample, this method of calculation often gives a very different result from that given by the method of approximation, i. e., by $\frac{mS}{R}$, in which the value of the blank is not taken into account.

As an illustration of an extreme case note the following data: Standard, 0.50 mg, average reading, 64; blank, 0, average reading, 90; no correction for error in tubes gives b=1.2 mg. Standard, 1.00 mg; average reading, 60; known sample, 0.50 mg, average reading, 79.75; no correction for error in tubes. Citral calculated by this method gives 0.46 mg, citral calculated by method of approximation gives 0.75 mg. The theoretical amount of citral present is 0.50 mg.

This is an extreme case, since the alcohol used in the test contained a large amount of aldehyde, and the difference between the readings of the standard and the sample was large, with the result that the aldehyde in the alcohol represented a large proportion of the whole quantity present in the sample. However, in the case of a preliminary test in which the citral content of the sample lies about halfway between two of the 1, 2, and 3 mg standards, the error introduced by ignoring the blank (assuming that the alcohol gave a blank of about 0.5 mg), would range from 2 to 11 per cent of the result, the variation depending on the amount of citral present. In the case of a sample of very low citral content the error would be very high, for example, in the case of the 0.5 mg sample, the error would amount to 34 per cent of the result, while much higher errors would be given by weaker samples (400 per cent error if 0.1 mg sample be compared by way of preliminary test with the 1.0 mg standard). This method of calculating results would therefore appear to be particularly indicated in passing from the preliminary to the final test and becomes almost a necessity when there is considerable difference between the readings of standard and sample and when using an "aldehyde-free" alcohol of the quality I was obliged to use.

Summing up briefly, I am inclined to be of the opinion that when care is taken to start sample and standard at the same time and to insure identical treatment throughout, the colorimetric work furnishes the chief source of error; there must be a number of readings taken, which requires time and results in more or less warming up of the solutions.

In order to introduce no error from this source the increase in depth of color of sample and standard must be in the same proportion.

The citral and the aldehyde in the blank appear to differ somewhat in their rate of developing color, but the rate of increase will presumably be identical for each if the quantity of citral be the same in each, since the quantity of the blank remains practically the same. The importance of having the standard and sample of nearly equal value in the final comparison is thus apparent.

Any method, then, of preliminary examination which will give the closest agreement between sample and standard for the final comparison is to be advocated. Such a method appears to be given by the scheme outlined, in which the value of the blank is estimated and made to enter into the calculation.

Averages of the results obtained by the provisional method after the methods for reading the colorimeter and for securing identity of treatment of samples and standard described above had been adopted, are as follows:

Sample No. 5 (4 tests, extremes 0.106 and 0.118), 0.111; No. 6 (3 tests, extremes 0.079 and 0.087), 0.082; No. 7 (7 tests, extremes 0.328 and 0.365), 0.347; No. 8 (4 tests, extremes 0.190 and 0.200), 0.194.

Several of the collaborators seemed to have some difficulty with the Hiltner method. This, however, was in all probability due to the fact that the method was a new one and very few had sufficient time to become thoroughly familiar with it. The results reported, however, are remarkably close and entirely satisfactory with the exception of sample No. 6, which contained cane sugar. The comments of collaborators on the Hiltner method were as follows:

E. R. Lyman reported that a blank omitting the Hiltner reagent gave appreciable color; therefore, the precaution was taken to run a blank in each case and correct the final figure accordingly. The method presented no difficulties and the color comparisons were unexpectedly consistent.

W. C. Burnet reported that the Hiltner method gave a great deal of trouble at first. It was found impossible to obtain results until after a week's work, but the results reported, except in the case of sample No. 6, were almost identical with those obtained by the provisional method. As the method was finally worked out, it was considered very simple and quick, and if the results obtained were accurate it was thought preferable to the provisional one. It was found absolutely necessary to use fresh reagents, however.

C. D. Howard finds that a correction for the color of 2 cc in connection with the Hiltner method amounts to approximately 0.03 per cent. This amount was not deducted from the results reported, however, as no correction was made for the color with the provisional method. The method is much more readily carried out by making preliminary trials by it, the necessity for such trials with the fuchsin-sulphite method would be obviated.

R. W. Hilts found that the color of the samples interfered in the Hiltner method. For preparing the reagents fuller's earth was much more satisfactory than animal charcoal; with the latter, the reagent became turbid and fluorescent in a few hours, while that clarified with fuller's earth remained reasonably clear for a day.

A. P. Sy reported that the results obtained by the Hiltner method varied considerably with the character of the extract, coloring matter, etc., and that it was quite difficult to compare samples with the standards, as the tints varied considerably. The alcohol available gave a strong reaction for aldehydes and interfered seriously with the results. He advises the use of aldehyde-free alcohol and the removal of the coloring matter.

W. A. Bender reported that final comparisons were made with amounts of extract containing about 2 mg of citral, 95 per cent alcohol being used throughout. The color corrections were made as follows: 10 cc of the extract was made up to 50 cc with alcohol in Hehner tubes. In the other tube were placed

2 cc of citral solution (equivalent to 2 mg) and 10 cc of the reagent, with enough alcohol to make up to 50 cc. The colors were then compared. The color from 10 cc of extract generally equaled that of 10 cc of the standard, giving a correction of 1 cc of standard for each cubic centimeter of extract solution.

H. Lewis Jackson reported that he was not able to get a "practically colorless" solution of the reagent. It was always of a greenish tint and quite dark, the color not being removed with fuller's earth or animal charcoal.

Mr. Hiltner, commenting upon his method, thinks that in the case of extracts strongly colored yellow, the results for citral would be too high, as is the case with sample No. 6. To ascertain the effect of the yellow tint he colored the standard citral solution with turmeric until the color was practically the same as that of the sample, and obtained almost identical results as with the uncolored standard.

Mr. Mory's comments were as follows:

There was no modification made of the Hiltner method further than to reverse the tubes in reading and to use the metaphenylene diamin hydrochlorid solution without clarifying, since with the reagent we have, a fairly colorless solution is given which is not improved by treatment with fuller's earth. Animal charcoal was found to be entirely unsuitable, giving a dark-colored solution which could not be improved by further treatment.

I am unable to explain the large difference found between the results obtained by the two methods on sample No. 6; the fact that the color produced by this sample changed very decidedly on standing overnight, becoming a muddy brown, while all the other samples remained yellow, suggests something abnormal about the sample.

The average obtained from two runs by the Hiltner method are for No. 5 (0.110 and 0.100), 0.105; No. 6 (0.148 and 0.141), 0.145; No. 7 (0.370 and 0.344), 0.352; No. 8 (0.209 and 0.194), 0.202.

It is interesting to note that the first set of these results by the Hiltner method, all of which are higher than the second set, were obtained by making up all of the standards and samples before reading any of them, with a resultant delay in making the comparisons, while the second or lower set of results were obtained by following more closely the method to the extent of making up and comparing immediately each sample with its standard.

Conclusions and Recommendations.

Some work was outlined for the removal of coal-tar dyes before the determination of citral by the provisional method. As was shown last year, the dye can not be removed by treatment with woolen cloth and acid without the destruction of considerable quantities of the aldehyde. The results, moreover, do not seem to be affected, and work along this line will be abandoned.

On the whole, it would seem that the work this year has accomplished the object for which it was designed. Objections to the use of a standard solution of citral derived from lemon grass, when the citral to be estimated is derived from lemon oil, have been effectively removed, as it has been shown that the results are accurate in either case. The objection that the presence of cane sugar materially affected the results when obtained by the provisional method is not substantiated, as the error, if there be one, is within the limit of the personal equation and is therefore negligible. It is also evident that within another year we shall have an additional method for the determination of citral in the Hiltner method, which would have been recommended for adoption except for the fact that the results are uniformly high where cane sugar is present. It does not seem, however, that this difficulty will offer any serious obstruction to the final adoption of the method, as it can undoubtedly be overcome. The preparation of aldehyde-free alcohol still causes considerable difficulty in some cases, and it is suggested that the use of Columbian spirit

be given a trial in the work in the future. It has been reported by at least one chemist, Mr. P. W. Tompkins, that he has successfully used this reagent without purification, the acetone present not affecting the reaction.

Only one recommendation is made, namely, that the provisional method for the determination of citral in lemon extract (Cir. 43, page 10, or Bul. 122, p. 32) be adopted as the official method.

The referee desires to bring before the association one other matter. It would seem desirable to adopt, in the near future, methods for the examination of the flavoring substances used in the preparation of extracts. A scheme for the analysis of lemon and orange oils is accordingly presented for consideration, and notice is given that the associate referee will ask for their provisional adoption at the next meeting of the association. While this subject has been treated in the Pharmacopœia, it would undoubtedly be a great benefit to have such methods adopted by the association. The methods proposed are as follows:

METHODS FOR EXAMINATION OF LEMON AND ORANGE OILS.

1. *Specific gravity.*

Determine the specific gravity by means of a pyknometer or a Sprengel tube at 15.6° C., as directed under XIII Wine, page 83, Bul. 107, Revised.

2. *Index of refraction.*

Determine the index of refraction with any standard instrument making the reading at 20° C.

3. *Rotation.*

Determine the rotation at 20° C. with any standard instrument using a 50 mm water-jacketed tube and sodium light. The results should be stated in angular degrees on a 100 mm basis. If instruments having the sugar scale are used, the reading on orange oils is above the range of the scale. With instruments provided with double compensation, readings may be obtained by setting forward the lævo reading scale to 50, taking the reading as usual and correcting by the addition of 50°. With single wedge instruments readings may be obtained by the use of standard lævo reading quartz plates.

4. *Determination of citral.*

Weigh into a small-stoppered flask a small quantity of the sample and dilute with aldehyde-free alcohol in proportion of 2 grams of lemon oil or 4 grams of orange oil to 100 cc of solution. Determine the citral by the fuchsin-sulphite method as directed on page 32 of Bulletin 122 or page 10 of Circular 43.

5. *Determination of the physical constants of the 10 per cent distillate.*[a]

Place 50 cc of the sample in a 3-bulb Ladenburg flask in which the main bulb has a diameter of 6 cm and is of 200 cc capacity and which has the condensing bulbs of the following dimensions: 3.5 cm, 3 cm, 2.5 cm, and in which the distance from the bottom of the flask to the opening of the side arm is 20 cm. Distil the oil at the rate of 2 cc per minute until 5 cc have been distilled. Determine the refractive index and rotation of this distillate as directed above.

6. *Detection of pinene.*

Mix the 10 per cent distillate as obtained above with 10 cc of ethyl nitrite and cool the mixture thoroughly in a freezing bath. Add slowly with constant shaking a mixture of 2 parts concentrated hydrochloric acid and 1 part of water which has been previously cooled. Keep the mixture in the freezing

[a] Schimmel and Co. Report, 1898, p. 41.

bath during this operation and allow it to remain therein for fifteen minutes. Filter off the crystals formed, using vacuum and washing with strong alcohol. Return the filtrate and washings to the freezing bath and allow them to remain for fifteen minutes. Filter off the crystals formed, using the original filter paper. Wash the two crops of crystals thoroughly with alcohol. Dry at room temperature and dissolve in the least possible amount of chloroform. Reprecipitate the nitroso-chlorid crystals with methyl alcohol and mount for examination under the microscope with olive oil. Pinene nitroso-chlorid crystals have irregular pyramidal ends while limonene nitroso-chlorid crystallizes in needle forms.[a]

7. Detection of alcohol.

Add to 5 cc of the oil a few drops of the following reagent and shake thoroughly. Alcohol is indicated by the formation of a blue color:

Reagent: 0.20 gram cobalt nitrate and 0.40 gram sulpho-cyanate of ammonium dissolved in 30 cc of water.

8. Determination of alcohol.

The amount of alcohol present in oils which have been used for the manufacture of terpeneless lemon extract may be approximately determined by washing repeatedly with small portions of water and determining the alcohol in these washings in the usual way.

DISTINCTION OF VANILLA EXTRACT AND ITS IMITATIONS.

By A. L. Winton and C. I. Lott.

Need of Proposed Method.

True vanilla extract is characterized by its vanillin content (which varies within limits yet to be determined), the presence of resins, the formation with normal or basic lead acetate of a flocculent gray brown precipitate, and the yellow color of the filtrate from the lead precipitate. Imitation vanilla flavor prepared from vanillin, with or without coumarin, and colored with caramel, may or may not contain an amount of vanillin within the limits for a true vanilla extract, depending on the formula used in its preparation, but in either case it is readily distinguished by the absence of resins, the scanty dark brown precipitate with normal or basic lead acetate, and the brown color of the filtrate from this precipitate. Qualitative tests for the characteristic constituents are, however, of uncertain value in the examination of mixtures, and there is obvious need of a reliable quantitative method which will enable an analyst to decide whether or not an extract is pure and, if adulterated, will furnish a basis for estimating the extent of the admixture.

The quantitative determination of resins, separated by dealcoholization, having proved unsatisfactory, our attention was directed to the determination of the lead number obtained by the use of normal or basic lead acetate. B. H. Smith, and also Jackson and McGeorge,[b] have used for the purpose the method devised by Winton [c] for the determination of the lead number in maple products. This method brings out striking distinctions, but the lead subacetate from a dealcoholized solution, in our experience, carries down with it a considerable amount of vanillin, thus precluding the possibility of determining vanillin, coumarin, and the lead number in one weighed portion. Normal lead acetate solution is known to be free from this objection and when substituted for the

[a] Drawings of these crystals are given in Circular 46 of the Bureau of Chemistry.

[b] J. Ind. Eng. Chem., 1909, 1:478.

[c] J. Amer. Chem. Soc., 1906, 28:1204.

basic acetate in the method gives results which, although much lower than those obtained by the ordinary process, serve well in differentiating the products.

The process thus modified permits the determination of vanillin in the aliquot of the filtrate, thus obviating the tedious filtration and washing of the precipitate, which is one of the serious disadvantages of the Hess and Prescott method. The filtration on the dry paper, as carried out in the proposed method, consumes but about fifteen minutes, whereas in the other process the washing of the precipitate usually requires a day. A description of the combined method follows:

Method as Modified.

Weigh 50 grams of the extract directly into a tared 250 cc beaker with marks showing volumes of 80 and 50 cc, dilute to 80 cc, and evaporate to 50 cc in a water bath kept at 70° C. Dilute again to 80 cc with water and evaporate to 50 cc. Transfer to a 100 cc flask, rinsing the beaker with hot water, add 25 cc of standard lead acetate solution (80 grams of normal lead acetate made up to one liter), make up to the mark with water, shake, and allow to settle for at least three hours, or preferably overnight. Decant on to a small dry filter and pipette off aliquots for the determination of vanillin, coumarin, and the lead number.

Vanillin and coumarin.—Remove 50 cc of the filtrate to a separatory funnel and determine vanillin and coumarin by the Hess and Prescott method.[a]

Normal lead number.[b]—To an aliquot of 10 cc of the filtrate from the lead acetate precipitate add 25 cc of water, a moderate excess of sulphuric acid and 100 cc of 95 per cent alcohol. Let stand overnight, filter on a Gooch crucible, wash with 95 per cent alcohol, dry at a moderate heat, ignite at low redness for three minutes, taking care to avoid the reducing flame, and weigh. The normal lead number is calculated by the following formula:

$$P=\frac{100\times 0.6831\ (S-W)}{5}=13.662\ (S-W)$$

in which P=normal lead number, S=grams of lead sulphate corresponding to 2.5 cc of the standard lead acetate solution as determined in a blank analysis, and W=grams of lead sulphate obtained in 10 cc of the filtrate from the lead acetate precipitate as just described.

Comparative Results.

In the following table are given analyses of seven vanilla extracts made by a reputable manufacturer from different kinds of beans, showing the percentages of vanillin obtained by the usual method, and by the methods combining the determination of vanillin and normal lead number, and the determination of vanillin and basic lead number, also the lead numbers given by normal and basic lead acetate. As the exact proportion of beans used in making these extracts and the methods of manufacture are not definitely known the results are presented as a study of the methods and not as data for determining limits of composition.

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[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. 107, Rev., p. 156.

[b] In order to avoid confusion, the words "normal" and "basic" are suggested as suitable for distinguishing the numbers obtained, respectively, by normal and basic lead acetate.

Results on authentic vanilla extracts by the proposed modifications.

Kind of bean.	Vanillin.			Lead number.	
	Usual Hess and Prescott method.	Combined method.		Normal.	Basic.
		Normal acetate.	Basic acetate.		
	Per cent.	*Per cent.*	*Per cent.*		
Mexican (Cut)[a]	0.14	0.16	0.12	0.61	1.06
Seychelles	.15	.17	.09	.35	1.00
S. American	.18	.18	.14	.50	1.13
Comores (Mayotte)	.24	.24	.12	.37	1.01
Tahiti	.09	.09	.03	.29	.99
Vanillon[a]	.09	.09	.08	.64	1.09
Bourbon		.20		.61	

[a] 12 per cent extract, all other samples are 10 per cent.

It will be noted that the amounts of vanillin by the proposed method, using normal acetate, agree closely with those by the usual Hess and Prescott process, with a tendency to slightly higher results, due partly to the volume occupied by the lead precipitate, but chiefly, it is believed, to the freedom from the error, due to imperfect washing of the precipitate, which is inherent to the usual process.

Using the basic acetate, as has been stated, the vanillin results are worthless.

The basic lead numbers (calculated to 10 per cent extract) are only about half those reported by Jackson and McGeorge, who evidently precipitated without dealcoholization, under which condition the resins, as well as certain water-soluble constituents, would form precipitates with the lead. Alcohol may also decrease the solubility of the precipitate.

The following results on normal lead numbers in three samples of commercial vanilla extract stated to be pure and in samples declared to be various solutions of vanillin and coumarin, or mixtures of such with vanilla or tonka extract, illustrate the practical application of the method:

	Lead numbers.
Commercial vanilla extract (1)	0.34
Commercial vanilla extract (2)	.34
Commercial vanilla extract (3)	.29
Vanilla, vanillin, tonka, and coumarin flavor (1)	.17
Vanilla, vanillin, tonka, and coumarin flavor (2)	.06
Vanilla, vanillin, and coumarin flavor	.10
Vanillin and coumarin flavor	.03
Vanillin flavor	.03
Imitation vanilla flavor	.10

Some experiments which we have made indicate that the lead numbers of mixtures are roughly proportional to the percentages of true vanilla extract present; if not, they can doubtless be made proportional by adding potassium sulphate, following the Ross modification of the lead process as applied to maple products (see page 58).

For the purpose of establishing limits for vanilla extract it is proposed to determine vanillin and the lead number on about 70 extracts which have been prepared from as many varieties or grades of beans in the laboratory.

At this point in the proceedings the Secretary of Agriculture briefly addressed the convention, referring to the importance of dif-

ferent phases of the work and the difficulties to be overcome by the chemist.

Mr. Edmund Clark, associate referee on baking powders, reported progress in the work along the following lines, no cooperative studies having been made on account of the early date of the meeting:

As a preliminary investigation, several samples of so-called calcium acid phosphate have been examined. It is well known that calcium monophosphate has during the past few years come to be generally used as an acid ingredient of baking powder. In the manufacture of this salt the phosphatic material is decomposed with sulphuric acid, the latter being afterwards neutralized with lime. This leaves a large residual of calcium sulphate, which may or may not be thoroughly removed in the purification of the product. Calcium monophosphate is deliquescent, and in order that it may keep dry some absorbent material is ordinarily mixed with it. This is customarily starch, though in many instances the calcium sulphate is not thoroughly removed and may be used as the drying ingredient. The results so far obtained indicate a wide variation in the content of calcium sulphate and starch in the commercial samples of "calcium acid phosphate" as it is sold upon the market. The analytical details will not be submitted until the study has been further pursued and cooperative work obtained.

Mr. Jaffa presented on behalf of Mr. T. Brailsford Robertson a paper entitled "A rapid method for the determination of the percentage of casein in milk." This paper may be found in full in the Journal of Industrial and Engineering Chemistry, 1909, volume 1, page 723.

REPORT ON SPICES.

By A. F. Seeker, *Associate Referee.*[a]

The associate referee on spices in his report at the last meeting expressed his dissatisfaction with the results of the cooperative work concerning the detection of added oil in paprika, and suggested that a further investigation of the subject be made this year. Accordingly the referee has endeavored to find a method by which different analysts can obtain concordant results, and to ascertain whether the iodin number of the nonvolatile ether extract may be relied upon to detect the presence of olive oil.

Having analyzed a large number of paprikas, it is the opinion of the present referee that the sample sent out last year was entirely abnormal and, being of unknown history, its purity is open to suspicion, and therefore conclusions based on its analysis should be accepted with caution. It seems safe to assume, however, that the radical difference in the results reported by the different analysts may be attributed in large measure to the method of determining the nonvolatile ether extract, and the present referee has accepted the suggestion of his predecessor by submitting some conventional methods for trial.

The work of Mr. Woods seems to indicate that the discrepancies in the results on last year's samples were largely due to a more or less complete extraction with ether. Apparently the extraction of 1 gram of material was not complete in 100 hours. Furthermore, the iodin number of the nonvolatile extract

[a] The referee wishes to acknowledge the assistance of W. A. Bender in the prosecution of this work.

steadily diminished as the amount of extract increased, so that with an extract of 5.43 per cent the iodin number was 82.0, and with 13.93 per cent the iodin number was 42.6. Such results appear to be possible only with a sophisticated sample, and to test this point further four different paprikas were extracted by the official method for crude fat, conducting the extraction for different lengths of time as specified in the following table:

TABLE 1.—*Increase in nonvolatile ether extract upon prolonged extraction and decrease in iodin number of the extract.*

[A and D were pure paprika, B and C contained added oil.]

Time of extraction in hours.	Nonvolatile ether extract.	Iodin number of nonvolatile ether extract.	Time of extraction in hours.	Nonvolatile ether extract.	Iodin number of nonvolatile ether extract.
Sample A:			Sample C:		
1	9.71	130.0	1	16.85	111.2
7	11.42	130.5	7	17.73	109.7
16	12.30	128.3	16	18.23	109.7
86	12.51	127.1	86	18.83	106.7
Sample B:			Sample D:		
1	15.10	111.6	1	13.53	126.9
7	16.42	110.7	7	14.39	127.1
16	17.41	109.1	16	14.69	126.6
86	17.62	106.4	86	15.24	122.7

The extraction of A and B was practically complete in sixteen hours, but the extract of C and D gained about 0.5 per cent upon further treatment. It is also noticeable that the iodin number of the ether extract diminishes as the amount of extract increases. These changes are by no means so marked in the samples under discussion as in those examined last year by Mr. Woods, and omitting the results obtained by the eighty-six hours' extraction, which is an impracticable time, the results furnish data which clearly indicate the nature of the samples.

To ascertain whether the difficulty in obtaining complete extraction lay in the presence of coarse particles, all four samples were passed through sieves of different degrees of fineness with the following results, the numbers at the heads of the columns referring to the number of meshes to the inch:

TABLE 2.—*Percentage of samples passing through sieves of varying degrees of fineness.*

Sample.	Under 40.	Between 40 and 60.	Between 60 and 80.	Between 80 and 100.	Over 100.	Total.
A	1.9	38.6	15.4	11.0	30.6	97.5
B	1.6	30.0	15.0	18.0	33.0	97.6
C	1.8	23.6	18.6	23.4	34.0	101.4
D	3.0	31.0	25.6	20.8	20.8	101.2

All appear to be fine enough to offer no difficulties to the usual mode of ether extraction, and the figures have additional interest in that they show how finely commercial paprikas are ground.

Three conventional methods appeared available for trial, and these were submitted to the collaborators to be employed on two samples of paprika of known history. Method I was that employed by Doolittle and Ogden.[a] Method

[a] J. Amer. Chem. Soc., 1908, **30**: 1481.

II was suggested in outline by A. L. Winton, certain details which appeared necessary from the volatility and high coefficient of expansion of the ether being added by the referee. In order to secure results by this method which would render the data obtained by Doolittle and Ogden available in interpreting analyses, a few preliminary trials were made upon two paprikas, one of which contained 4 per cent of added oil and the other none. The paprika was allowed to stand in contact with the solvent for different lengths of time, the analyses showing that two hours gave results closely agreeing with those obtained by the method of Doolittle and Ogden.

TABLE 3.—*Preliminary test of Methods I and II (paprika two hours in contact with solvent).*

Method and sample.	Nonvolatile ether extract.	Iodin number of nonvolatile ether method.
Pure paprika:	*Per cent.*	
I	13.32	133.1
II	13.41	133.1
Paprika containing 4 per cent added oil:		
I	17.50	119.5
II	17.31	119.5

The three methods in detail are as follows, Method III having been suggested by L. M. Tolman.

Method I.—Two grams of paprika are brushed into a 9 cm closely woven filter and dried in a sulphuric acid desiccator for at least twelve hours. The paper and its contents are then placed in a funnel and washed with exactly 200 cc of anhydrous alcohol-free ether prepared as directed on page 39, Bul. 107, Revised. The filtrate is collected in a tared (air-dried) glass-stoppered flask which has been counterpoised against a similar flask. The capacity should be 250 to 300 cc. Keep the funnel covered during the filtration and when all the ether has run through distil off the solvent. Disconnect the flask as soon as the ether ceases to drop from the condenser and dry in a steam or water oven, laying the flask on its side to allow the ether vapor to escape. Dry for thirty minutes, then cool for thirty minutes in the air, and weigh. Repeat the heating and cooling until the weight is constant to less than 1 mg. Report percentage of ether extract so obtained.

Dissolve the ether extract in the flask with 10 cc of chloroform and when solution is complete add 30 cc of Hanus solution, following the method on page 137, Bul. 107, Revised, allowing thirty minutes for the halogen absorption. Report iodin number of the ether extract.

Method II.—Four grams of paprika are spread on a watch crystal and dried over sulphuric acid for at least twelve hours. Two hundred cubic centimeters of anhydrous alcohol-free ether (Bul. 107, Revised, page 39) are measured into a graduated glass-stoppered flask on which the graduation is placed near the lower part of the neck. Brush the paprika into the flask and place a mark on the neck at the place where the meniscus now is, stopper, and shake thoroughly at twenty-minute intervals during two hours. Before shaking for the last time note whether the level of the meniscus has changed; and if so, bring it back to the mark on the flask by adding the necessary amount of ether if the level has fallen, or by cooling in water if the level has risen. Allow the solid matter to sink, and carefully decant 100 cc of the supernatant liquid into a graduated flask. Filter this 100 cc through a closely woven 11 cm filter into a tared (air-dry) 250 to 300 cc glass-stoppered flask that has been counterpoised against a similar flask. Wash the filter with anhydrous alcohol-free ether until the last portions of extractive matter have been removed, and distil off the ether. As soon as the solvent ceases to flow from the condenser remove the flask and place it on its side in a steam or water oven for thirty minutes. Cool the open flask in the air for thirty minutes and weigh. Repeat the heating and cooling

in the same manner until the weight is constant to less than 1 mg. Report the percentage of ether extract so obtained.

Dissolve the ether extract in the flask with 10 cc of chloroform and when solution is complete add 30 cc of Hanus solution, following the method on page 137, Bul. 107, Revised, and allowing thirty minutes for the halogen absorption. Report the iodin number of the ether extract.

Method III.—Ten grams of paprika are spread in a thin layer on a flat-bottomed dish and dried for two hours in a vacuum oven at 60° C. and 25 mm. In the absence of a vacuum oven dry for twenty-four hours in a sulphuric acid desiccator. The material is then transferred to a double filter and washed with 300 cc of cold anhydrous alcohol-free ether. After distilling off the ether the residue is taken up with fresh ether and filtered into a small tared beaker, the filter paper being carefully washed with ether to remove all traces of oil. After again evaporating off the ether the residue is dried to constant weight at 100° C. After the final weighing the residue is washed with chloroform into a 100 cc flask and made up to volume with this liquid. Determinations of the iodin number are made on 10 cc portions of this solution. Use anhydrous alcohol-free ether prepared as directed, page 39, Bul. 107, Revised. For the iodin number use Hanus solution and follow the method given on page 137, Bul. 107, Revised. Report percentage of ether extract and iodin number of the ether extract.

General precautions.—If on distilling off the ether the extract becomes colorless, or nearly so, reject it, as the determination for the iodin number will invariably be found too low. This is caused by impure ether and the latter must in this case be again purified. Glass vessels of 250 to 300 cc capacity, owing to their large surface, require at least thirty minutes' cooling in the air before weighing.

Before submitting these methods to the collaborators, ten samples of paprika were examined under the direction of the referee by all three procedures and also by the official method of determining crude fat. Samples 1, 8, 9, and 10 were Hungarian paprika; 2, 3, 4, 5, 6, and 7 were Spanish paprika. Number 10 was the same as number 9 except that it contained 4 per cent of added oil. The analytical results are given in Table 4.

TABLE 4.—*A comparison of four different methods for determining the nonvolatile ether extract of paprika, with the iodin numbers of the extracts.*

NONVOLATILE ETHER EXTRACT.

Methods.	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.	No. 7.	No. 8.	No. 9.	No. 10.
Official method	12.30	14.12	17.41	13.36	18.23	17.37	17.04	14.69	14.89	18.44
Method I	9.69	12.33	15.08	11.54	16.75	15.62	15.38	13.49	13.74	17.50
Method II	10.57	13.04	15.64	12.12	17.44	15.98	15.75	13.85	14.06	17.46
Method III	10.03	12.55	15.22	11.81	15.73	15.68	15.43	13.69	13.07	17.20

IODIN NUMBER OF EXTRACT.

Official method	128.3	121.5	109.1	126.2	109.7	109.8	110.3	126.6	129.5	118.4
Method I	129.8	123.6	111.8	128.5	111.4	111.6	111.7	127.0	133.6	119.5
Method II	128.8	122.0	110.3	126.8	111.6	111.5	111.7	127.3	133.8	119.6
Method III	134.1	124.8	113.1	128.2	114.4	115.5	114.0	130.4	136.4	123.6

A study of this table shows that all three conventional methods fall far short of complete extraction, the differences between the amount of nonvolatile extract obtained by each, as compared with the official method for crude fat, are as follows: Method I, 0.94 to 2.61, average 1.67; Method II, 0.79 to 1.77, average 1.20; and Method III, 1.00 to 2.50, average 1.74. Method II yields the largest amount of ether extract, and Methods I and III about equal amounts, but less than Method II.

It will be observed also that the iodin number of the ether extracts obtained by the official method is invariably lower than that of the extracts in the conventional methods. This further corroborates the previous work and shows that upon long-continued extraction of the paprika some substance having a small iodin absorption appears to pass into the extract, lowering its iodin value.

In general the iodin numbers obtained by Method III are higher than those obtained by any of the other methods, this being in part due to the cause just mentioned, but doubtless also to the fact that in comparison with the other methods about half as much extract is employed in the determination of the iodin number, leaving a much larger excess of halogen.

Upon the whole, all four methods check each other excellently as far as the detection of added oil is concerned, not one of the 40 values giving a false indication as to the nature of the product. It was therefore decided to submit the three conventional methods to the collaborators, in order to obtain their opinions and to ascertain which procedure would yield the most concordant results.

Whole paprika pods, obtained from some European dealers by Mr. R. E. Doolittle, were ground under the direction of the referee, shells and seed being ground together, rejecting only the stems and placentæ. The seeds were included for the reason that commercial samples as a rule contain them, though the Standards of Purity for Food Products (Circular 19, Office of the Secretary) prescribe that only the shells shall be used.

Owing to lack of sufficient material of one kind it was found necessary to mix two parts of Hungarian with one part of Spanish pods. The whole was thoroughly mixed and divided into two parts, one being sent out as "Paprika A, untreated," the other being treated with 5 per cent of olive oil, which was evenly incorporated with it, and the product then sent out as "Paprika B, containing 5 per cent of added oil."

Sixteen chemists volunteered to cooperate on spices, and seven reports were received, the names of those taking part being: (1) D. B. Bisbee, Bureau of Chemistry, St. Louis, Mo.; (2) A. T. Collins, The A. Colburn Company, Philadelphia, Pa.; (3) R. W. Hilts, Bureau of Chemistry, Philadelphia, Pa.; (4) E. C. Hull, Bureau of Chemistry, New York, N. Y.; (5) C. I. Lott, Bureau of Chemistry, Chicago, Ill.; (6) H. E. Sindall, Weikel & Smith Spice Company, Philadelphia, Pa.; and (7) C. P. Wilson, Bureau of Chemistry, Washington, D. C.

The results obtained by these analysts are given in Table 5.

TABLE 5.—*Cooperative results on paprikas of known history by three methods.*

Analyst and method.	Pure paprika.		Paprika containing 5 per cent of olive oil.	
	Nonvolatile ether extract.	Iodin number of ether extract.	Nonvolatile ether extract.	Iodin number of ether extract.
METHOD I:				
1	10.08	130.74	14.63	114.03
	10.13	130.74	14.60	114.02
2	9.64	129.1	13.97	117.6
	9.65	134.86	14.00	117.4
	9.63	131.08		
3	9.82	134.5	14.34	115.6
	9.81	134.7	14.22	115.9
4	9.38	135.7	14.13	116.9
	9.47	136.9	13.82	119.4
5	9.94	129.43	14.47	113.53
	9.92	129.69	14.52	111.79

TABLE 5.—*Cooperative results on paprikas of known history by three methods*—Continued.

Analyst and method.	Pure paprika.		Paprika containing 5 per cent of olive oil.	
	Nonvolatile ether extract.	Iodin number of ether extract.	Nonvolatile ether extract.	Iodin number of ether extract.
METHOD I—Continued.				
6	9.59	126.7	14.03	114.4
7	9.84	128.16	14.99	104.9
	9.80	127.89	14.98	105.01
Minimum	9.38	126.7	13.82	104.9
Maximum	10.13	136.9	14.99	119.4
Difference	.75	10.2	1.17	14.5
Average	9.77	131.45	14.36	113.89
METHOD II:				
1	10.10	131.36	.....	.....
2	10.70	131.71	14.65	113.97
	10.07	124.76	14.33	116.0
3	9.90	133.8	14.42	116.1
	9.81	136.0	14.31	116.5
4	10.66	133.1	13.91	118.0
	10.45	133.1	14.74	117.5
5	10.25	129.54	14.75	113.22
	10.34	124.68	14.76	112.90
6	9.60	130.8	14.19	114.1
7	10.13	127.59	14.54	112.04
	10.22	123.98	14.54	111.63
Minimum	9.60	123.98	13.91	111.63
Maximum	10.70	136.0	14.76	118.0
Difference	1.10	12.02	.83	6.37
Average	10.18	130.04	14.46	114.72
METHOD III:				
1	9.96	132.6	14.45	117.14
2	9.70	128.66	14.03	114.89
	.....	135.2	.....	114.89
	.....	128.66	.....	.....
3	9.64	138.5	14.14	119.6
	.....	138.5	.....	119.6
4	9.13	136.5	13.80	122.4
	9.24	140.0	13.79	120.0
5	9.69	132.28	14.11	116.94
	.....	132.28	.....	116.49
6	9.37	128.3	13.96	116.9
7	9.78	132.72	14.44	114.06
	9.85	132.17	14.46	116.53
Minimum	9.13	128.3	13.79	114.06
Maximum	9.96	140.0	14.46	122.4
Difference	.83	11.7	.67	8.34
Average	9.59	133.57	14.13	117.45

A review of the results reported by the six analysts shows that the iodin number of the ether extract clearly distinguishes between the pure paprika and the one treated with olive oil. Of the 33 iodin numbers reported on the extract of the pure paprika only two are lower than the minimum for a pure product. The two values in question are only three units below the minimum limit. All of the iodin numbers obtained in the case of the sample treated with olive oil indicate the sophistication.

Excepting a few determinations in each series the results in general are concordant. To arrive at a fair judgment on this point it may be assumed that the average of all the results reported in each series is the correct one. Then by

taking the difference between this and each individual result and averaging the differences, a figure representing the average error is obtained. Proceeding in this way the average errors in the three methods are as follows:

TABLE 6.—*Variations from the average by the three methods.*

Method.	Pure paprika.		Paprika containing added oil.	
	Nonvolatile ether extract.	Iodin number ether extract.	Nonvolatile ether extract.	Iodin number ether extract.
	Per cent.		*Per cent.*	
I	0.18	2.76	0.31	3.03
II	.24	3.08	.21	1.87
III	.23	3.23	.22	1.99

It is obvious that on the score of accuracy there is little choice between the methods, and the one offering the greatest ease of manipulation may be selected. The results also corroborate those of the referee in the preliminary trials (Table 3), the averages showing that Method III gives higher iodin values than the other two, although all three clearly indicate the nature of the sample.

COMMENTS OF THE COLLABORATORS.

D. B. Bisbee prefers Method II, because in the other procedures it is difficult to exhaust the ether-soluble matter from the paprika. He attributes the higher iodin values of Method III to the greater excess of halogen used.

A. T. Collins finds by experiment that in decanting the aliquot part of ether in Method II there is considerable evaporation, depending in amount upon temperature and air currents. He proposes to take the aliquot by means of a pipette. Proceeding in this way he obtained in the pure paprika an extract of 9.66 per cent, with an iodin number 134.03. He also found that the variation in iodin numbers was not caused by difference in temperature, nor did the keeping of the reaction mixture in the dark have any apparent influence.

R. W. Hilts secured the aliquot of the ether solution in Method II by fitting the maceration flask with an attachment like a wash bottle and blowing 100 cc into a graduated flask. He considers that Method III does not secure a complete extraction of the sample with ether, and that the higher iodin numbers are caused by the larger excess of halogen. Employing double the amount of chloroform solution prescribed he obtains an iodin value of 135.1 for the pure paprika. He prefers Method II for simplicity of manipulation and because it involves less personal attention and smaller possibilities of difference in manipulation. He recommends that the amount of material be increased to 5 grams and the volume of solution to 250 cc so that duplicates may be determined in one operation.

E. C. Hull prefers Method I for simplicity of manipulation, and thinks that Method II may be improved by pipetting the aliquot of ether solution instead of decanting, because the evaporation of solvent in this operation is variable and at times amounts to 2 or 3 cc. He considers that Method III involves too much manipulation.

C. I. Lott proposes a fourth method which is similar to Method II, but differs in that the volume of ether used is only 100 cc, the whole mixture of paprika and solvent being filtered after standing thirty minutes, and an aliquot of 50 cc taken from the filtrate with a pipette. His results on the two paprikas were: Sample A, extract 10.31 per cent, iodin number 132.35; sample B, extract 15.11 per cent, iodin number 116.37.

H. E. Sindall prefers Method II, and suggests that it be modified so that larger amounts of paprika are taken and a proportionate amount of ether, making it possible to run duplicates by one extraction.

C. P. Wilson prefers Method I for simplicity of manipulation, and suggests that Method III could be improved by taking 20 cc of the chloroform solution

instead of 10 cc in the determination of the iodin number, thus reducing the percentage of error.

Conclusions.

(1) The iodin number of the nonvolatile ether extract as determined by the three conventional methods described in this report may be employed to distinguish pure paprika from that treated with olive oil.

(2) Methods I and II are to be preferred for simplicity of manipulation. Method II may be improved by taking 5 grams of paprika and employing 250 cc of ether for the extraction, using a pipette for taking aliquots of 100 cc in the determination of the nonvolatile extract, thus making it possible to run duplicates on the iodin number of the extract by a single extraction.

(3) The official method for the determination of crude fat, besides consuming more time, will probably yield less concordant results in the hands of different analysts, owing to the difficulty of obtaining complete extraction of some samples with ether. Long continued extraction of a sample seems to cause a lowering of the iodin number of the extract.

The referee suggests that this work be continued, selecting one of the conventional methods and employing it on a number of samples of known history. A further study of the ether extract of pure paprika should be undertaken with a view to securing a confirmatory test for added oil and also to discover some means whereby the presence of an oil having an iodin number approaching that of paprika extract could be detected.

REPORT ON MEAT AND FISH.

By F. C. Weber, *Associate Referee.*

The first report on this subject which was made last year gave the results obtained from the analysis of chicken meat by the methods in common use for the separation of protein nitrogen, the object being to show how soon and how accurately these methods will show deterioration of meats. It was found that the increase in the water-soluble total nitrogen and the increase in ammonia agreed fairly well with the macroscopical and physical appearance of the samples, but the methods as employed were not sufficiently delicate to detect the slight changes and incipient deterioration which take place in meats on standing. The object of the work of this year was to find a chemical method which would indicate with greater accuracy these changes.

After a great deal of experimental work a method was evolved which in many respects is similar to one reported, since the inception of this investigation, by Scala and Bonamartini.[a] These investigators have also discarded the separation of the nitrogenous constituents of meats as being valueless as far as determining incipient deterioration is concerned. Their results, however, are on meats which have been allowed to stand at room or ice box temperature for from twenty-four to seventy-two hours.

It is quite evident that the method as here described, since it is sufficiently delicate to detect changes occurring in meats after standing in cold storage (at a temperature ranging from 6° above to 6° below 0° F.), will answer equally well for conditions more favorable to decomposition. The method as finally adopted was as follows:

Weigh 25 grams of finely ground meat or fish into beakers of 100 cc to 150 cc capacity, add 50 cc pepsin-hydrochloric acid solution (1 gram of pepsin

[a] Annali Ig. Sper., 1909, No. 1, pp. 113–122.

and 1 cc of hydrochloric acid to 500 cc of water), and, after stirring the contents of the beaker thoroughly till all the larger particles of meat are broken place in an incubator at 37.5° C. for one and one-half to two hours. After incubation transfer to an 850 cc Erlenmeyer flask with 300 cc of water, add 10 to 15 grams of light magnesium oxid, free from sulphur and arsenic, and distil through a vertical condenser into an approximately hundredth-normal iodin solution for one hour, so arranging the gas flame that 200 cc distils over during this time. Loss of iodin by volatilization is guarded against by cooling the receiving flask, sealing the joints with paraffin and attaching four U tubes, each containing 5 cc of hundredth-normal sodium thiosulphate solution. The contents of the receiving flask and U tubes are poured into an Erlenmeyer flask and the excess of iodin titrated with hundredth-normal sodium thiosulphate, using starch as an indicator. A blank should be run at the same time, using the same amount of water and magnesium oxid. It is also well to run a control on a sample of absolutely fresh meat.

The results are reported as cubic centimeters of hundredth-normal iodin required for 25 grams of meat.

The average of a number of determinations on chicken meat, using the method as above outlined, required 29.6 cc of hundredth-normal iodin solution for absolutely fresh meat and for ground chicken meat which had been in storage seven months 38.8 cc were required.

Fresh beef required 26.8 cc, while beef which had been in storage nearly two years required 34.1 cc of hundredth-normal iodin solution.

The average for absolutely fresh eggs, using in this case only 15-gram samples, was 19.3 cc, while eggs stored for a period of only seven months required 26.7 cc of hundredth-normal iodin solution.

From the data which have been collected so far it would appear that this method can be utilized for testing the age of meats by chemical determinations, and it is recommended that the referee for next year give the method as outlined in this report a thorough trial with various kinds of meats.

REPORT ON FATS AND OILS.

By T. J. Bryan, *Referee.*

Directions and Samples.

The work undertaken this year has been the determination of fish oils in vegetable oils. The method suggested was for both the qualitative and quantitative detection of fish oil in the presence of vegetable oils, as devised by Eisenschiml and Copthorne. It depended upon the fact that bromids formed by the action of bromin on fish oil are insoluble in a hot mixture of equal parts of glacial acetic acid and chloroform. Work done by the referee showed that the quantitative method for the determination of bromids was in itself very satisfactory. It is well known, however, that the amount of the bromids formed from the same fish oil vary with the age of the oil, the change being probably due to oxidation. It was not expected, however, that different samples of the same kind of oil would show such a wide variation as was found in the actual work done by the quantitative method. Some samples of common menhaden oil contain as low as 9 per cent of bromids by this method, others as high as 26 per cent, rendering the quantitative determination of the amount of insoluble bromids formed absolutely worthless for ascertaining the amount of fish oil present in any mixture with vegetable oil. The qualitative method suggested, however, being based on this definite chemical reaction promises to be of great value. Former methods in present use are uncertain and unreliable, are not based on

a known, definite chemical reaction and do not show the presence of fish oil in small amounts. It was therefore thought advisable to determine the value of this qualitative method.

The directions sent to the collaborators were as follows:

Dissolve in a test tube about 3 grams of the oil in 6 cc of a mixture of equal parts of chloroform and glacial acetic acid. Add bromin drop by drop until a slight excess is indicated by the color, keeping the solution at about 60° C. Allow to stand fifteen minutes or more and then place the test tube in boiling water. If only vegetable oils are present, the solution will become perfectly clear, while fish oils will remain cloudy or contain a precipitate due to the presence of insoluble bromids.

Ten samples were sent containing the following mixtures:

(1) Linseed oil + 10 per cent of crude menhaden oil;
(2) Linseed oil + 5 per cent of crude menhaden oil;
(3) Olive oil + 10 per cent of cod-liver oil (domestic);
(4) Olive oil + 5 per cent of cod-liver oil;
(5) Olive oil + 10 per cent of extra bleached winter menhaden oil;
(6) Peanut oil + 10 per cent of light pressed menhaden oil;
(7) Peanut oil + 5 per cent of light pressed menhaden oil;
(8) Peanut oil + 5 per cent of Norwegian cod-liver oil;
(9) Cotton-seed oil + 10 per cent of bleached winter menhaden oil;
(10) Cotton-seed oil + 5 per cent of bleached winter menhaden oil.

Results of Cooperative Work.

It will be observed that none of the samples were pure vegetable oils. The collaborators were requested to apply the method given not only to the ten samples, but also to any samples of vegetable oils they might have in their laboratory. The following reports on these samples were received from seven collaborators:

Fish oil reported as present (+) or absent (0) in official samples.

Analyst.	Number of sample and amount of fish oil present.									
	1. 10 per cent.	2. 5 per cent.	3. 10 per cent.	4. 5 per cent.	5. 10 per cent.	6. 10 per cent.	7. 5 per cent.	8. 5 per cent.	9. 10 per cent.	10. 5 per cent.
H. S. Bailey	+	+?	+	+	+	+	+	+	+	+
G. C. Spencer	+	+	+	+	+	+	+	+	+	+
R. W. Hilts	+	+	+	+	+	+	+	+	+	+
P. Rudnick[a]	0	0	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
F. W. Liepsner	+	?	+	0	+	+	0	0	+	+
T. C. Pinketon	+	+	+	0	0	+	0	0	+	0
A. Lowenstein	+	+	+	0	+	+	0	0	0	0

[a] Used double quantities of both oil and solvents for second series of determinations.

Comments by the Collaborators.

Herbert S. Bailey: A sample of olive oil which had been standing in the laboratory for several months was used as a blank and gave little or no precipitate on bromination and was perfectly clear in boiling water.

G. C. Spencer: I think the test is easier to manipulate if the bromin is added to the cold oil solution, as the heat of the action rapidly warms it up and it is less violent and less apt to cause excessive frothing.

R. W. Hilts: I also tested the following vegetable oils: Sweet almond oil, cocoanut oil, palm oil, olive oil, peanut oil, and sunflower seed oil. All these remained perfectly brilliant, showing no insoluble compound whatever.

P. Rudnick: In addition to these samples we tested a sample of cotton-seed oil, which gave absolutely no precipitate with bromin either hot or cold.

While it is out of the question to comment on methods when working entirely with unknown samples, as in this instance, it seems to me that the test worked rather better with larger proportions than those given, or at any rate with an increased proportion of chloroform.

Thomas C. Pinketon: I have reported traces in Nos. 6 and 9. The indication is very slight, and for commercial work I would be inclined to report these samples as not adulterated with fish oil.

A. Lowenstein: Negative results were obtained with cotton-seed oil, peanut, raw linseed, and bean oil.

Comments of the Referee.

The results, in the opinion of the referee, are very promising, as four of the seven collaborators obtained correct results on all samples. Two of the others, as shown by their reports, concluded that no fish oil was present in some cases, though they were satisfied that the hot chloroform acetic acid solutions were not perfectly clear.

It will be noted that the referee sent out only samples containing fish oil, though it has been customary to submit some solutions containing none of the substance which the method sought to detect. Probably the conclusions would have been accurate in more cases had such samples been sent.

Further work in the referee's laboratory [a] has shown that the recommendation of Mr. Rudnick is of value, and that when larger quantities are used of both solvent and oil, the test becomes more certain. It has also been found that the bromination may be carried on at any temperature without varying the results, hence it is most convenient from every point of view to use room temperature. With these modifications the test is believed to be reliable in the detection of fish oil when present to the extent of 5 per cent. The greatest present value of this test would appear to lie in the detection of fish oil in raw linseed oil, but it is thought that it may prove to be of use for other purposes also. It is recommended that the work on this qualitative test be continued another year.

REPORT ON THE ADULTERATION OF DAIRY PRODUCTS.

By Hermann C. Lythgoe, *Associate Referee.*

A study has been made of the Baier and Neumann method [b] for the detection of added sugar in cream and of the relation between the fat, the alkalinity of the ash, and the calcium in milk and cream. Cooperative work has been done by my assistant, Mr. Clarence E. Marsh, by Prof. James O. Jordan and Mr. Frank E. Mott, of the milk inspection department of Boston, Mass., and also by Mr. Harry E. Barnard and Mr. William D. McAbee, of the Indiana state board of health.

It has been claimed that pure milk will give the Baier and Neumann test. Occasional samples of pure milk will give a pale blue color, but this can be entirely removed by filtration, and the filtrate will be green while the color due to sugar will pass through the filter, giving the usual blue solution characteristic of adulterated samples. The color produced is due to a reduction of the molybdic acid and is produced by levulose and dextrose as well as by sucrose. Solutions of 1 gram of lactose, levulose, dextrose, and sucrose in 35

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[a] B. C. Gardner, assistant state analyst, is to be credited with much of the analytical work on which this report is based.

[b] Zts. Nahr. Genussm., 1908, **16**: 51; U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 52.

cc of water were used in comparing the amount of color produced when heated with the molybdenum reagent for five minutes. Lactose produced no color, levulose gave a heavy blue, sucrose a weaker blue, and dextrose the weakest blue corresponding in intensity as 10 : 3 : 1.

Stannous chlorid and ferrous sulphate give this blue color, but the reaction takes place in the cold and in small quantities the color disappears on heating. In order for the color to persist after heating the sample of cream must contain these substances to the extent of 1 per cent calculated as the metal. In this case the sample will be completely coagulated and the taste would be very disagreeable. Hydrogen sulphid will also give the blue color, but it will disappear on heating. If the solution does not show the blue color before heating, it is free from hydrogen sulphid, ferrous sulphate, and stannous chlorid.

As a confirmatory test for sugar the resorcin test may be applied to the serum prepared with uranium, as described in the method, or to the cream. This test is given by sucrose and levulose, but not by dextrose or lactose.

Messrs. Barnard and McAbee report that "the Baier and Neumann test was applied to all of the samples of known-purity cream, and in no case did it react positively until the calcium sucrate was added. This test was also applied to about 60 samples of milk and cream which were collected by the inspectors of this department, and gave a negative reaction for every sample." Mr. Jordan says: "My opinion of the Baier and Neumann test, based upon repeated trials and much investigation, is that it is efficient, and may be safely relied upon as a test for cane sugar in cream." The experience of the referee also shows that the test is reliable, and this opinion is based on the examination of 245 samples of cream collected on the Massachusetts market, several samples of known-purity cream, and about 100 samples of known-purity milk, together with numerous samples of cream separated in the laboratory from samples of market milk, all of these samples giving a negative reaction by the test as described.

The graphic charts (figs. 3 to 6) show the relation between the fat, the calcium content, and the alkalinity of the ash in cream samples. In the pure-cream charts the samples marked with a cross were known purity, the samples having been milked and separated in our presence, the samples marked with a triangle were commercial samples, giving a negative reaction with the Baier and Neumann test, and the samples marked with a circle were separated in the laboratory from milk collected by the inspectors, all of the cream samples giving negative reactions for sugar. Professor Jordan and Mr. Mott report the following figures for fat and calcium oxid in known-purity cream. These figures are below the critical line of the chart.

Fat.	Calcium oxid.
Per cent.	*Per cent.*
50	0.080
46	.095
44	.095
37	.180
35	.125

Last year the referee made a study of various methods for the preparation of milk serum but no change in the provisional method was recommended. This year an attempt was made to secure a method having the advantages of

the asaprol citric-acid method of Baier and Neumann [a] without its defects. As a result of this attempt the following method has been devised by the referee.

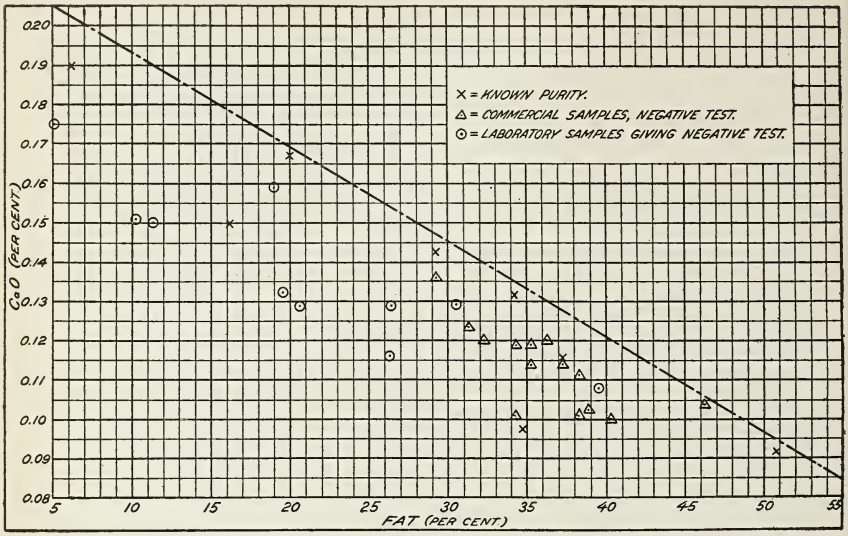

FIG. 3.—Relation between fat and calcium (CaO) in pure cream.

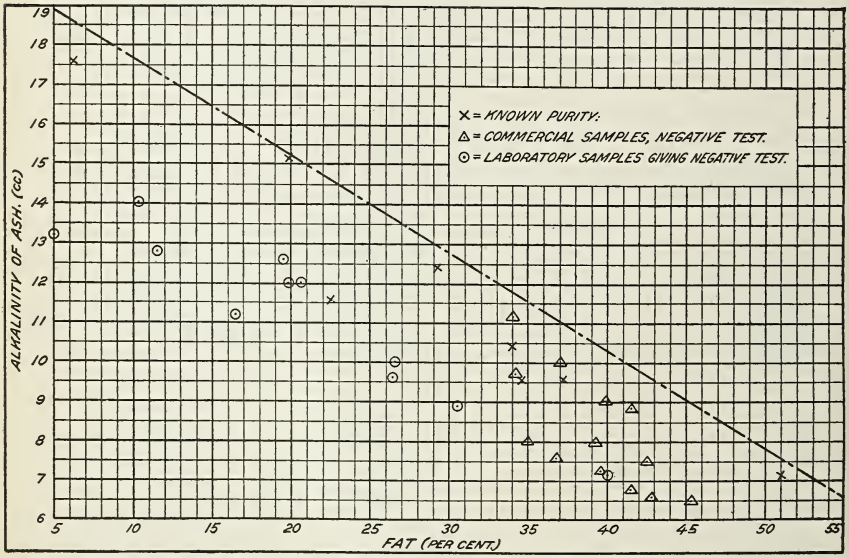

FIG. 4.—Relation between fat and alkalinity of ash in pure cream.

Dissolve 72.5 grams of crystallized copper sulphate in water and dilute to 1 liter. If this solution does not refract at 36 on the scale of the immersion

[a] Nahr. Genussm., 1907, **13**: 369; U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 51.

refractometer at 20° C. add water or copper sulphate until the desired result is obtained. To 8 cc of the copper solution add 32 cc of milk, shake well, and

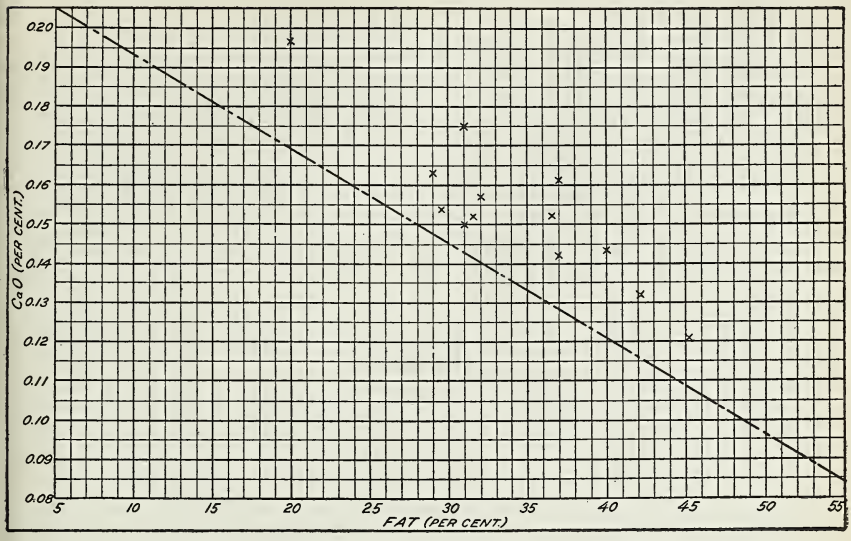

FIG. 5.—Relation between fat and calcium (CaO) in adulterated cream.

pour upon a filter. When the filtrate begins to come through clear, change the receiver, pour the small quantity of cloudy filtrate upon the filter and continue

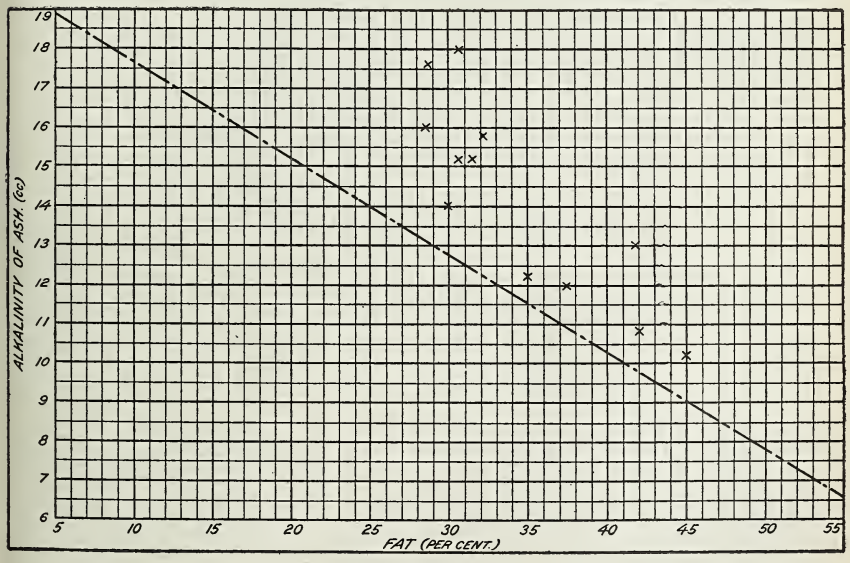

FIG. 6.—Relation between fat and alkalinity of ash in adulterated cream.

the filtration as usual. Refract the clear filtrate at 20° C. by means of the Zeiss immersion refractometer. A reading below 36 indicates added water.

The advantages of this method over the acetic-acid method are as follows: It is quicker; heating of the sample is unnecessary, consequently there is no error due to evaporation; the variation in refraction of pure milk is less; 10 per cent of added water will reduce a high-grade milk below the minimum, whereas 15 per cent is required in the case of the acetic-acid method.

The following tables show the analyses of 150 samples of milk of known purity, 14 of which are herd milk and the rest from individual cows. It will be seen from these analyses that milk of the widest range in composition has been used in this investigation. The total solids varied from 17.17 to 10.40 per cent, the fat from 7.70 to 2.46 per cent and the solids not fat from 10.05 to 7.50 per cent. The refraction of the copper serum varied from 36.1 to 39.5 while in some of the same samples the variation in the refraction of the acetic acid serum ranged from 39.5 to 45.5. These copper refractions are distributed as follows:

Refraction 39.0 to 39.5, 6 samples; 38.0 to 38.9, 66 samples; 37.0 to 37.9, 65; and 36.1 to 36.9, 13 samples. The herd milk gave refractions as follows: Highest, 38.6; lowest, 37.2; and average, 37.9.

A composite sample of laboratory milk was divided into two portions, one portion watered (25 per cent), sera prepared, and analyses made with the following results:

Analysis of a composite sample of laboratory milk, whole and watered.

Determinations.	Whole milk (per cent).	Watered milk (25 per cent).	Determinations.	Whole milk (per cent).	Watered milk (25 per cent).
Total solids	12.08	9.06	Composition of serum—Con.		
Fat	3.50	2.65	Total solids	6.21	4.85
Solids not fat	8.50	6.41	Milk sugar	4.32	3.23
Composition of serum:			Proteids	.59	.39
Specific gravity (15° C.)	1.0280	1.0234	Ash	.83	.79
Refraction (20° C.)	37.3	32.6	Copper	.21	.25

Analysis of milk having varying percentages of added water.

Added water.	Total solids.	Fat.	Solids not fat.	Refraction of copper serum at 20° C.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
0	13.60	4.65	8.95	38.1
10	12.24	4.18	8.06	35.8
20	10.88	3.72	7.16	33.8
30	9.52	3.25	6.27	32.0
40	8.16	2.79	5.37	30.1
50	6.80	2.33	4.47	28.3

The following samples of milk of known purity and source were analyzed in the referee's laboratory with the assistance of Messrs. Hickey, Nurenberg, and Marsh. W. G. Tice, of the New Jersey State board of health, has also submitted some results on pure and watered milks which are appended. The variation in the composition of the milk samples is from 15.89 to 10.46 per cent in total solids, from 6.60 to 2.35 per cent in fat, from 10.23 to 7.62 per cent in solids not fat, and from 36.0 to 39.7 in refraction. The refraction figures are distributed as follows: Refraction number 39.0 to 39.7, 8 samples; 38.0 to 38.9, 15 samples; 37.0 to 37.9, 24 samples; and 36.0 to 36.9, 6 samples.

Analysis of milk of known purity.

[Massachusetts board of health.]

JERSEY COWS.

Age.	Time since calving.	Weight of milk.	Specific gravity (15° C.).	Total solids.	Fat.	Proteids.	Ash.	Solids not fat.	Milk sugar.	Refraction of copper serum (20° C.).
Years.	*Months.*	*Pounds.*		*Per ct.*	*Per ct.*	*Per cent.*	*Per ct.*	*Per ct.*	*Per ct.*	
2	5	6	1.033	17.17	7.70	3.65	0.72	9.42	5.05	39.5
.....	7	11	1.032	16.16	6.80	3.25	.75	9.80	5.80	38.0
5	3	20	1.033	16.00	7.20	2.79	.81	8.80	5.20	39.2
.....	2	12	1.032	16.00	6.80	3.88	.72	9.20	4.60	37.1
7	6	8	1.034	15.56	6.10	3.81	.80	9.46	4.85	38.2
5	7	10	1.035	15.52	6.20	3.36	.81	9.32	5.15	38.9
2	5	8	1.034	15.21	5.60	4.09	.72	9.61	4.80	38.1
7	7	8	1.033	15.12	6.00	3.33	.69	9.12	5.10	38.9
8	3	8	1.033	15.10	6.00	3.33	.77	9.10	5.00	38.6
3	6	8	1.032	14.66	6.30	3.06	.80	8.36	4.50	38.0
7	6	6	1.036	14.56	5.00	3.58	.83	9.56	5.15	38.5
4	4	12	1.033	14.34	5.00	3.79	.75	9.34	4.80	38.7
7	6	8	1.035	14.15	5.10	3.47	.78	9.05	4.80	38.4
7	6	8	1.033	14.10	5.40	2.99	.76	8.10	4.95	38.6
.....	3	11	1.032	13.56	4.65	3.57	.64	8.91	4.70	38.4

GUERNSEY COWS.

5	8	3	1.034	16.33	6.40	4.63	0.82	9.93	4.48	37.5
5	8	5	1.035	15.83	5.95	4.06	.77	8.88	5.05	38.4
10	15	5	1.035	15.80	5.75	4.24	.73	10.05	4.98	38.8
7	8	3½	1.034	15.66	5.80	3.99	.77	9.86	5.10	38.4
17	15	2½	1.034	15.55	5.60	4.58	.79	9.95	4.58	37.9
5	4	8	1.034	15.50	6.20	3.60	.71	9.30	4.99	37.7
4	8	7	1.034	15.35	5.70	3.72	.71	9.65	5.22	38.5
10	17	8	1.034	14.90	5.30	4.03	.76	9.60	4.81	38.4
3	6	7	1.033	14.40	5.20	3.68	.72	9.20	4.80	38.8
4	6	9	1.033	14.32	5.00	3.77	.75	9.32	4.80	38.1
4	14	8	1.032	14.14	5.20	3.43	.72	8.94	4.80	38.4
5	8	3	1.034	13.99	4.60	3.66	.70	9.39	5.03	39.0
6	15	5	1.031	13.26	4.65	3.53	.73	8.61	4.75	37.3
4	1	12	1.033	13.08	4.25	2.96	.73	8.83	5.14	38.1
7	14	7	1.032	12.92	4.00	3.61	.70	8.92	4.61	37.0
8	1	12	1.032	12.52	4.35	2.65	.77	8.17	4.75	38.0
4	7	11	1.032	12.22	3.80	2.98	.75	8.43	4.70	37.4
8	11	8	1.031	12.15	4.15	2.26	.69	8.00	5.05	37.4

GRADE AYRSHIRE COWS.

.....	2	13	1.032	15.32	6.00	3.94	0.73	9.32	4.65	38.8
.....	2	16	1.031	14.00	5.10	3.60	.70	8.90	4.60	38.6
.....	.....	7½	1.032	13.70	4.80	3.55	.80	8.90	4.55	37.7
5	2	14	1.033	13.54	4.55	3.39	.74	8.99	4.86	38.2
8	10	4	1.031	13.36	4.60	3.13	.72	8.76	4.91	37.9
4	8	6	1.032	13.32	4.35	3.07	.69	8.97	5.21	37.4
7	8	6	1.032	13.28	4.30	3.86	.67	8.98	4.45	38.3
.....	.....	13½	1.031	13.22	4.80	2.85	.77	8.42	4.80	37.8
.....	.....	9	1.032	13.13	4.80	2.75	.83	8.33	4.75	37.0
.....	.....	6	1.032	13.08	4.60	2.88	.75	8.46	4.85	37.8
.....	.....	11	1.032	13.04	4.60	3.27	.82	8.44	4.35	37.4
4	7	8	1.032	12.72	4.00	3.46	.73	8.72	4.50	38.2
.....	.....	14	1.030	12.36	4.60	2.11	.85	8.76	4.80	38.5
10	½	12	1.031	12.17	3.70	3.46	.76	8.47	4.20	37.3
12	4	9	1.032	11.90	3.30	3.04	.70	8.60	4.65	36.4
7	2	16	1.030	11.78	3.80	2.44	.77	7.98	4.74	37.4
7	3	.....	1.030	10.77	3.00	2.45	.68	7.77	4.64	37.0

Analysis of milk of known purity—Continued.

GRADE JERSEY COWS.

Age.	Time since calving.	Weight of milk.	Specific gravity (15° C.).	Total solids.	Fat.	Proteids.	Ash.	Solids not fat.	Milk sugar.	Refraction of copper serum (20° C.).
Years.	*Months.*	*Pounds.*		*Per ct.*	*Per ct.*	*Per cent.*	*Per ct.*	*Per ct.*	*Per ct.*	
8	15	2	1.033	15.28	5.65	4.20	0.78	9.63	4.55	37.6
7	8	8	1.033	15.18	5.80	3.53	.79	9.38	4.80	38.2
7	8	8	1.032	14.40	5.40	3.69	.86	9.00	4.45	37.6
.....	.....	8	1.032	14.00	5.40	3.05	.70	8.60	4.85	38.2
3	7	5	1.031	13.98	4.80	3.46	.70	9.18	5.02	37.6
6	4	6	1.034	13.59	4.35	3.30	.75	9.24	5.19	37.9
7	8	8	1.034	13.45	4.20	3.47	.77	9.45	5.05	37.6
9	2	14	1.033	12.80	3.85	3.10	.72	8.95	5.15	38.3
7	8	.....	1.031	11.40	3.00	2.78	.71	8.40	4.91	37.5

GRADE GUERNSEY COWS.

4	4	6	1.034	15.45	5.70	3.79	0.75	9.75	4.90	37.9
7	1	13	1.034	14.74	5.20	3.94	.78	9.54	5.00	39.0
4	7	.....	1.033	14.22	5.00	3.50	.69	9.22	5.03	38.9
7	8	12	1.034	14.03	4.30	3.88	.78	9.73	4.90	38.9
8	8	12	1.031	13.22	4.80	2.88	.66	8.42	5.10	38.1
9	7	14	1.032	13.14	4.60	2.99	.77	8.54	4.90	38.0
8	1	11	1.035	13.03	3.80	3.66	.74	9.23	5.05	38.8
.....	.....	8	1.032	12.92	4.60	2.77	.75	8.32	4.80	37.9
7	9	6	1.033	12.88	4.00	3.35	.76	8.88	4.77	37.0
8	2	12	1.032	12.73	4.00	3.11	.73	8.33	4.85	37.9
7	7	10	1.032	12.56	4.10	2.98	.68	8.46	5.05	38.1
8	8	10	1.032	12.60	4.00	2.79	.75	8.60	5.15	38.2
8	8	13	1.032	11.82	3.80	2.43	.69	8.02	4.90	37.2
6	1	14	1.030	11.46	3.50	2.50	.66	7.96	4.80	37.3

SWISS COWS.

7	6	6	1.031	14.57	5.05	3.81	0.76	9.52	4.95	38.0
8	10	5	1.034	14.08	4.10	4.05	.74	9.98	5.19	38.5
4	7	10	1.032	13.70	4.65	3.14	.68	9.05	5.19	38.3
7	10	12	1.031	13.36	4.70	2.97	.68	8.66	5.01	37.7
4	4	8	1.034	13.30	4.30	3.46	.73	9.00	5.00	37.4
4	6	8	1.031	13.06	4.05	3.01	.67	9.01	5.33	37.7
5	5	10	1.032	12.94	4.00	3.19	.68	8.94	5.07	38.0
10	7	10	1.033	12.90	3.80	3.17	.70	9.10	5.25	38.4
4	5	7	1.031	12.66	4.00	3.18	.68	8.66	4.70	37.3
5	4	7	1.031	12.14	3.80	2.63	.70	8.34	4.75	37.4

GRADE HOLSTEIN COWS.

[a]5	7	8	1.034	14.51	4.90	3.81	0.74	9.61	5.09	38.6
[a]5	7	7	1.032	14.24	4.90	3.84	.72	9.34	4.81	38.3
7	10	7	1.030	14.50	5.40	3.48	.76	8.10	4.10	37.2
7	4	10	1.031	13.40	4.35	3.12	.74	9.05	5.19	37.9
4	2	14	1.031	12.40	3.60	3.06	.70	8.80	4.84	37.3
7	1	17	1.032	11.83	3.10	3.05	.68	8.73	5.05	37.8
4	1	.....	1.030	11.70	3.40	2.49	.66	8.30	5.15	38.0
3	7	10	1.032	11.68	3.40	2.85	.66	8.28	4.60	37.3
7	2	7	1.032	11.68	3.20	3.13	.71	8.48	4.50	37.0
5	2	20	1.032	11.35	3.00	2.67	.79	8.35	4.50	37.3
6	5	16	1.032	11.76	3.15	2.80	.71	8.61	4.80	37.2
4	1½	17	1.032	11.24	3.30	2.74	.68	7.94	4.50	37.0
5	5	16	1.030	10.94	3.30	2.54	.71	7.64	4.50	36.5
5	5	.....	1.029	10.70	3.20	2.33	.70	7.50	4.47	36.1

AYRSHIRE COWS.

.....	.....	8½	1.032	13.80	5.00	2.90	0.80	8.80	5.10	38.2
.....	.....	7½	1.032	13.35	4.50	3.03	.72	8.85	5.10	38.7
.....	.....	10½	1.030	12.07	3.90	2.35	.82	8.17	5.00	37.6
.....	.....	14	1.032	12.07	3.55	2.68	.77	8.45	5.00	38.3

[a] Twin cows.

Analysis of milk of known purity—Continued.

DUTCH BELT COWS.

Age.	Time since calving.	Weight of milk.	Specific gravity (15° C.).	Total solids.	Fat.	Proteids.	Ash.	Solids not fat.	Milk sugar.	Refraction of copper serum (20° C.)
Years.	*Months.*	*Pounds.*		*Per ct.*	*Per ct.*	*Per cent.*	*Per ct.*	*Per ct.*	*Per ct.*	
.....	.....	.....	1.033	14.09	4.75	3.47	0.70	9.34	4.85	39.0
.....	.....	.....	1.033	13.78	4.35	3.50	.76	9.43	5.17	38.5
.....	.....	.....	1.034	12.82	3.60	3.10	.73	9.22	4.90	39.0
.....	.....	.....	1.033	12.46	3.50	3.09	.66	8.96	4.75	38.6
.....	.....	.....	1.032	12.32	3.70	2.76	.72	8.62	5.10	38.7
.....	.....	.....	1.032	11.98	3.50	2.58	.66	8.48	5.10	38.5
.....	.....	.....	1.032	11.96	3.50	2.76	.70	8.48	4.90	38.5
.....	.....	.....	1.030	11.84	3.40	2.79	.71	8.44	4.75	38.0
.....	.....	.....	1.032	11.80	3.40	2.58	.70	8.40	5.00	38.4
.....	.....	.....	1.031	11.78	3.60	2.74	.66	8.18	4.65	37.7
.....	.....	.....	1.031	11.77	3.40	2.97	.70	8.37	4.70	38.2
.....	.....	.....	1.031	11.62	3.60	2.74	.70	8.02	5.05	38.3
.....	.....	.....	1.031	11.44	3.40	2.60	.72	8.04	4.90	38.0
.....	.....	.....	1.031	11.28	3.00	2.46	.69	8.28	5.05	38.6
.....	.....	.....	1.030	11.05	3.20	2.67	.74	7.85	4.50	37.3

HOLSTEIN COWS.

3	½	20	1.034	13.96	4.35	4.30	0.76	9.61	4.82	37.9
2	9	8	1.032	12.08	3.20	3.19	.73	8.88	5.16	37.6
4	7	6	1.032	12.00	3.30	3.22	.70	8.70	4.78	37.0
2	6	10	1.031	11.87	3.30	3.25	.70	8.57	4.85	36.7
4	1	17	1.032	11.82	3.60	2.90	.78	8.22	4.80	37.5
10	9	11	1.030	11.80	3.20	3.03	.75	8.60	4.84	36.5
4	10	.....	1.032	11.72	3.10	2.88	.66	8.62	5.08	38.2
4	6	11	1.032	11.58	3.30	2.81	.70	8.28	4.77	37.2
4	6	6	1.031	11.52	3.40	3.18	.72	8.12	4.22	36.2
6	8	12	1.030	11.38	3.70	2.59	.71	7.68	4.40	36.8
4	2	16	1.031	11.31	3.15	2.73	.75	8.16	4.68	37.2
4	7	10	1.030	11.26	3.00	2.89	.68	8.28	4.69	36.8
4	1	16	1.032	11.18	2.80	2.64	.68	8.38	5.00	37.9
5	½	17	1.031	10.95	2.90	2.63	.73	8.05	4.89	37.3
6	1	[a] 24	1.032	10.78	2.80	2.66	.74	7.98	4.58	36.4
		[b] 26	1.031	10.78	2.70	2.68	.73	8.18	4.80	36.3
		[c] 29	1.030	10.62	2.55	2.42	.75	8.07	4.90	36.3
3	15	9	1.030	10.65	2.75	2.70	.67	7.90	4.53	36.1
3	10	.....	1.029	10.60	3.00	2.61	.72	7.60	4.27	36.2
7	½	15	1.031	10.40	2.75	2.70	.76	7.65	4.19	36.1

[a] Noon milking. [b] Night milking. [c] Morning milking.

Mixed milks.

[Massachusetts board of health.]

Designation of breed.[a]	Specific gravity. (15° C.).	Total solids.	Fat.	Proteids.	Ash.	Solids not fat.	Milk sugar.	Refraction of copper serum (20° C.).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
1	1.034	14.57	5.40	3.54	0.78	9.17	4.85	38.5
2	1.034	14.40	5.00	3.77	.77	9.40	4.86	38.6
3	1.033	13.40	4.25	3.31	.78	9.15	4.90	38.6
4	1.031	13.08	4.75	2.65	.68	8.33	5.00	37.7
5	1.031	12.95	4.00	3.09	.68	8.95	5.18	37.7
6	1.032	12.92	3.90	3.41	.76	9.02	4.75	37.9
7	1.032	12.62	3.85	3.34	.73	8.77	4.60	37.2
8	1.032	12.57	4.00	2.90	.77	8.57	4.90	38.1
9	1.032	12.56	4.20	2.83	.73	8.36	4.80	37.9
10	1.032	12.24	3.65	2.97	.70	8.59	4.82	37.6
11	1.032	12.18	3.80	3.00	.75	8.38	4.70	37.9
12	1.031	12.15	3.60	3.01	.70	8.59	4.92	37.7
13	1.032	12.03	3.60	2.62	.68	8.43	5.00	38.5
14	1.032	11.96	3.35	2.99	.69	8.61	4.89	37.2

[a] Character of herds from which mixed milk was obtained: 1. Jersey; 2. Guernsey; 3. Grade Durham, Grade Guernsey, Grade Hereford, Grade Holstein; 4. Guernsey and Grade Guernsey; 5. Swiss, Grade Ayrshire, Grade Holstein, Grade Jersey; 6. Grade Jersey, Grade Guernsey; 7. Swiss, Grade Holstein, Grade Ayrshire, Grade Jersey; 8. Ayrshire; 9. Ayrshire, Grade Jersey; 10, 11, 12. Holstein, Grade Holstein, Grade Guernsey, Grade Jersey; 13. Dutch Belt; 14. Holstein and Grade Holstein.

Composition of milk of known purity.

[Wm. G. Tice, New Jersey.]

Breed.	Specific gravity (15° C.).	Total solids.	Fat.	Solids not fat.	Refraction of serum (20° C.).		Weight of milk.
					Acetic.	Copper.	
		Per cent.	*Per cent.*	*Per cent.*			*Pounds.*
Jersey	1.0305	15.98	6.60	9.38	43.0	38.0	12
Do	1.0290	14.76	6.20	8.56	41.8	37.5	20
Guernsey	1.0310	14.54	5.20	9.34	43.0	38.0	12
Jersey	1.0290	14.20	5.20	9.00	42.3	37.9	16
Guernsey	1.0290	13.70	5.00	8.70	41.0	37.0	16
Grade Jersey	1.0290	13.00	4.70	8.30	40.6	36.7	12
Grade Guernsey	1.0310	12.66	3.80	8.86	41.0	36.5	16
Grade Jersey	1.0300	11.88	3.30	8.58	42.4	37.9	20
Grade Guernsey	1.0350	11.04	2.70	8.34	41.3	37.4	30
Holstein [a]	1.0330	13.98	4.50	9.49	43.3	38.0	10
	1.0320	14.88	4.65	10.23	.....	38.6	12
Do	1.0300	13.90	4.10	9.80	.....	38.2	9
	1.0315	13.67	4.85	8.82	43.55	39.0	10
Jersey	1.0310	13.55	5.00	8.55	42.6	38.0	12
	1.0310	13.25	4.50	8.75	42.5	38.0	13
Holstein	1.0310	12.80	3.80	9.00	42.0	37.6	8
	1.0300	13.72	4.90	8.92	41.3	37.6	9
Jersey and Guernsey	1.0320	12.62	4.40	8.22	42.8	38.3	10
	1.0330	13.44	4.40	9.04	43.4	39.0	12
Do	1.0327	12.92	4.00	8.92	43.6	39.0	12
	1.0330	13.18	4.40	8.78	43.9	39.3	13
Holstein	1.0320	12.92	3.70	9.22	43.4	39.0	16
	1.0330	13.17	4.10	9.07	43.5	39.0	16
Do	1.0310	12.57	4.00	8.57	42.5	38.0	19
	1.0320	13.14	3.95	9.19	42.9	38.3	21
Do	1.0290	12.98	4.90	8.08	41.5	37.8	8
	1.0289	12.93	4.50	8.43	42.0	37.4	10
Do	1.0310	12.33	3.80	8.53	41.1	37.2	10
	1.0300	12.67	4.25	8.42	.....	37.3	12
Do	1.0305	12.14	4.00	8.14	42.0	38.2	12
	1.0298	12.42	4.10	8.32	41.8	39.7	14
Do	1.0305	12.14	4.00	8.14	42.0	38.2	12
	1.0298	12.42	4.10	8.32	41.8	39.7	14
Do	1.0310	12.12	4.60	7.62	41.6	37.6	12
	1.0298	12.42	4.20	8.22	41.5	37.3	13
Do	1.0305	11.74	3.32	8.42	41.0	37.4	12
	1.0300	12.53	4.00	8.53	42.1	38.1	14
Do	1.0305	11.79	3.30	8.49	41.2	37.5	14
	1.0298	12.48	4.25	8.23	41.7	38.4	12
Do	1.0285	11.01	3.10	7.91	39.8	36.0	12
	1.0290	12.36	3.70	8.66	41.6	37.3	13
Do	1.0305	11.99	3.60	8.39	41.0	37.5	13
	1.0310	12.18	3.50	8.61	.....	37.9	16
Do	1.0290	11.32	3.90	8.32	39.6	36.6	12
	1.0290	12.17	4.05	8.12	41.0	37.3	13
Do	1.0310	11.52	3.80	7.72	41.2	37.7	18
	1.0295	12.02	4.05	7.97	41.7	37.9	18
Do	1.0295	10.94	2.90	8.04	41.5	37.3	12
	1.0290	11.55	3.75	7.80	40.5	37.3	17
Do	1.0285	11.92	3.90	8.02	40.3	36.5	10
	1.0310	10.46	2.35	8.11	40.7	36.5	16
Herd milk	1.0300	12.20	3.60	8.60	41.6	37.7	.....
	1.0300	12.54	3.40	9.14	42.1	38.0	.....

[a] Bracketed data represent morning and evening milk in each case.

Analysis of whole milk systematically watered.

[Wm. G. Tice, New Jersey.]

Added water.	Total solids.	Fat.	Solids not fat.	Ash.	Specific gravity 15° C.).	Refraction of serum (20° C.).	
						Acetic.	Copper.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*			
0	12.40	4.15	8.25	0.62	1.0295	41.1	37.4
5	11.73	3.90	7.83	.60	1.0270	39.4	36.2
10	11.26	3.70	7.56	.53	1.0260	38.5	35.4
20	10.00	3.15	6.85	.44	1.0230	35.5	33.4
30	8.67	2.90	5.77	.40	1.0200	32.7	31.6

Recommendations.

It is recommended that—

(1) The Baier and Neumann test as modified be adopted as provisional.

(2) More work be done on the methods for the determination of the alkalinity of the ash and of calcium.

(3) The copper method of preparation be further studied by the association.

PRESIDENT'S ADDRESS.

By W. D. Bigelow.

Gentlemen of the Association: We are told that in the early days of the steam railroad stops were made at certain mileposts, where the engine was oiled and adjusted and the entire train inspected. The improvement of railroad equipment has lessened the frequency of such inspections, but in detail and thoroughness they have been increased.

The annual meetings of this association are the points at which we stop to inspect our equipment and to make such adjustment, repairs, and improvements as may be necessary or advantageous to further progress. At this, our twenty-sixth annual meeting, we are encouraged to feel that much of our early crudeness has been corrected, but it is no less inspiring to find that each stage of progress reveals a greater field of labor and to know that the development of this association is commensurate with its responsibilities.

The association has been frequently congratulated on its success in handling the questions that first received its attention. These questions, however, can not yet be laid aside as completed. Nor is it likely that we shall ever see them settled to our entire satisfaction. The total nitrogen, potassium, and phosphorus in a fertilizer, or, in fact, in any material, organic or inorganic, of simple or complex composition, can be determined with a reasonable degree of accuracy. The determination in a fertilizer of the availability of those elements to plants or to any particular plant will always tax to the utmost every faculty and facility at our command. Nor can we hope to settle this question permanently. As long as commercial conditions vary, as long as the agricultural conditions or practices of localities continue to change, as long as manufacturing processes continue to yield new products or by-products of fertilizing value, so long will the question of determining the availability of fertilizers continue to present new difficulties.

The work of the association that has attracted the most attention has been of a collaborative nature. By this means the greatest progress has been made in the improvement of analytical methods. The important chemical work involved in the majority of our technical studies consists in the application of these methods. Such methods are essential in all chemical investigations and too much care can not be devoted to their improvement. At the same time there is need in many directions of a greater amount of individual research in the study of agricultural products.

We believe that an intelligent comparison can be made of different samples of one particular product by means of the Wende methods. For instance, by such an analysis we can form an opinion of the composition and nutritive value of a sample of oats as compared with other samples of oats. It is possible, however, that our premises are not entirely tenable when from the same data we attempt to compare the composition or the nutritive value of oats with those of cotton-seed meal, corn, or hay. These products could be compared

with each other more intelligently if we understood better the indefinite groups of bodies now classed together as nitrogenous constituents, crude fiber, ether extract, and nitrogen-free extract. A detailed and exhaustive study of each of the general types of agricultural products may add much to the meaning of the results obtained by the routine methods commonly employed. It is probable that a considerable part of the energy now spent in securing results by the Wende methods might be diverted advantageously to a study of the groups determined by these methods. As an illustration of this, two analyses, recently obtained by the Bureau of Chemistry in the study of a cactus from Texas, are quoted. By the regular methods of the association the plant was found to contain 14 per cent of sucrose and 2.8 per cent of reducing sugar. Owing to the fact that the plant grows without cultivation and can be gathered at slight expense, it was suggested as a material for the manufacture of denatured alcohol. Its further study by C. S. Hudson, however, discloses the fact that the material hydrolyzed by hydrochloric acid was not sucrose. On hydrolyzing with a preparation of invertase he found that the plant contained but 0.70 per cent of sucrose and 13.3 per cent of a levulose glucosid readily hydrolyzed by dilute hydrochloric acid. It is not improbable that a more detailed study of our common agricultural products would disclose some unexpected compounds in the complexes ordinarily determined and thus advance our efficiency.

Many of the methods and standards of the association are recognized by the laws of the various States and all of them have the highest standing in the courts. In the Federal Food and Drugs Act and in the drug laws of the various States the U. S. Pharmacopœia is recognized. This association is therefore vitally interested in the accuracy and completeness of the analytical methods of the Pharmacopœia. Every ten years a new edition of this work is issued under the direction of a convention composed of delegates from "Incorporated Medical Colleges, and Medical Schools connected with Incorporated Colleges and Universities; Incorporated Colleges of Pharmacy, and Pharmaceutical Schools connected with Incorporated Universities; Incorporated State Medical Associations; Incorporated State Pharmaceutical Associations * * *. Delegates appointed by the Surgeon-General of the United States Army, the Surgeon-General of the United States Navy, and the Surgeon-General of the United States Marine-Hospital Service, and by the organizations not hereinbefore named, which were admitted to representation in the convention of 1900, shall also be members of the corporation."

I have requested that the Association of Official Agricultural Chemists be admitted to membership in the Pharmaceutical Convention which will meet next spring. If this request be approved by five members of the board of trustees it will be published in medical and pharmaceutical journals and finally submitted to the vote of the convention. Anticipating favorable action, I recommend that the president be authorized to appoint three delegates to this convention.

The scope of the association, so narrow at first, has been broadened from time to time as new subjects have demanded our attention. Starting with a single committee charged with the formulation of methods for the analysis of fertilizers, the work of the association has been subdivided and increased until now 51 referees and associate referees are required to direct our study of methods of analysis.

The nature of the organization has been correspondingly changed. From a simple organization dealing with a few subjects with which all members were familiar and in which all were vitally interested we have gradually developed

into a complex organization with a diversified field of work—dealing with subjects so great in number and so different in nature that no one member can be acquainted with them all intimately. This is in keeping with the degree of specialization demanded by the tendencies of the age. With this increasing complexity the association has wisely taken precautions against precipitate action. These safeguards are inconvenient, but they are necessary. The last one to be adopted becomes operative for the first time at the present meeting. I refer to the standing committee to receive three weeks in advance of the annual convention the recommendations of referees, consider them in advance of the meeting, and report them to the association. This is a wise provision, but it requires the cooperation of 60 men—51 referees and associates and the 9 members of the committee. It reaches further than that. It requires the cooperation of all the collaborators of all the referees. This cooperation is difficult to secure, but its importance is obvious. Our effort to secure it should start, not at the end of the year, but at the beginning.

It is more important than ever before that samples and directions for collaborative work be distributed at the earliest possible moment. It is equally important that collaborators undertake their work early and report results promptly. This is often inconvenient and sometimes impossible. All have "busy days" and "rush seasons," and the majority have periods when work is least pressing during which they plan to undertake such collaborative work. Yet I think it is our common experience that work that is laid aside for a "convenient season" is often never taken up and rarely receives the attention we had planned. Moreover, many of our samples undergo gradual change, and analyses made at intervals of six months or more are not always comparable.

Again, the results obtained from collaborators often show the importance of additional work, and when they are tabulated the referee not infrequently finds that further attention to certain points would make his report much stronger. It is my experience that when samples and directions are distributed with a request for results within a certain specified time—say a month or six weeks—fully as many reports are received as when no time is specified. This leaves time for additional work either by the referee or by collaborators and sometimes discloses imperfections in the method under examination which can be studied further.

Above all it should be borne in mind that the reports of referees must now be completed a month before the date of the meeting. The committee can not consider the referees' recommendations intelligently without the data on which they are based. A copy of the report must necessarily accompany the recommendations—or rather let us say an abstract of the report, as brief as possible, containing merely the data which the committee needs. And when this abstract is prepared why not bring it to the meeting and read it here instead of the full report? We listen sometimes for an hour, an hour and a half, or longer to a paper beginning with the correspondence with collaborators, giving in full a long and complex method or several of them in all their details, going into every particular of a whole year's work, including columns of figures so long that no human mind can grasp their significance. And when the reading of the report is over we have missed or forgotten every point it contained. Its reading has only conveyed one idea—that we would have profited by and enjoyed a fifteen-minute abstract.

As I have already stated, the association has found it wise from time to time to adopt safeguards to insure the proper conduct of business and prevent precipitate action. Some of these have been included in the constitution and are accessible. Others are only printed in the Proceedings. They have never

been compiled. Probably nobody knows them all. Possibly some of them have been forgotten. I would recommend that a committee be appointed to compile, in the form of by-laws or otherwise, the regulations of the association for the conduct of its business.

The constitution has been amended from time to time as the scope of the association has increased. It does not provide, however, for the subjects in which there has been the greatest activity during recent years—methods for the examination of foods and standards for the interpretation of the results of those methods. Neither does it provide for methods for the examination of medicinal plants and drugs, which has been undertaken more recently. I would suggest that these subjects be included. The constitution provides that "Only such chemists as are connected with institutions exercising official fertilizer control shall vote on questions involving methods of analyzing fertilizers." At the present time there are more institutions represented here that exercise food control than are charged with the enforcement of fertilizer laws. A constantly increasing number of our members are responsible for the enforcement of laws regulating the adulteration of drugs. Should not this provision of the constitution be broadened to include those subjects?

Among the difficulties that have grown with the scope of the association is the appointment of referees. It has not been found possible in recent years to obtain reports on all subjects covered by the association. Considering the large number of referees it would be strange if some were not unable to undertake the work they feel their subject demands. Yet each should attempt to make some progress. We should bear in mind the aggregate gain to the association that would result if each referee could improve a single detail of the methods of his subject each year. Our most conspicuous progress in the methods for the examination of a new class of products is made in the first year of our work upon them, when the crude methods are blocked out and first made workable. In the perfection of the methods, which often requires years of careful investigation, our progress is less evident but not less important. It is often this stage of our work that requires the greatest experience and the highest degree of skill.

FRIDAY—AFTERNOON SESSION.

The report on canned vegetables by W. L. Dubois, associate referee, consisted in the application of certain methods to the examination of fresh and canned peas, primarily with a view to distinguishing soaked goods. The analytical data obtained, however, proved to be of less value for this purpose than for the comparison of commercial grades. The full details of the study are to be found in Bureau of Chemistry Circular No. 54.

REPORT ON TEA, COFFEE, AND COCOA PRODUCTS.

By A. G. Woodman, *Associate Referee.*

Instructions and Cooperative Results.

A study of the Dubois method for the estimation of sucrose and lactose in milk chocolate (Bulletin 107, Revised, page 256), and a comparative test of the provisional and the Görter methods for the determination of caffein,

constituted the work for this year. Eight collaborators requested samples and results were received from four.

COFFEE.

The samples for analysis were accompanied by the following directions:

Place the sample as soon as received in a stoppered bottle, and grind the portions used for analysis through a 0.5 mm sieve, as described on page 152, Bulletin 107, Revised.

Follow the provisional method for caffein as given on page 153, Bulletin 107. After the extraction with chloroform is apparently complete, shake the exhausted residue with water and extract the water solution with further portions of chloroform. Examine this second extract separately to see how much much, if any, caffein it contains. Weigh the extracted caffein in each case and also determine it from the nitrogen content.

The Görter method (Annalen, 1908, **358**: 327) is conducted as follows:

Moisten 11 grams of the finely-powdered coffee with 3 cc of water, allow to stand for half an hour, and extract for three hours in a Soxhlet extractor with chloroform. Evaporate the extract, treat the residue of fat and caffein with hot water, filter through a cotton plug, and wash with hot water. Make up the filtrate and washings to 55 cc, pipette off 50 cc, and extract four times with chloroform. This chloroform extract is evaporated in a tared flask and the caffein dried at 100° and weighed.

Calculate the caffein from the nitrogen content also.

The results obtained are given in the following table:

Cooperative results on the determination of caffein in coffee by two methods.

Analyst.	Provisional method (N x 3.464).		Total.	Görter method.
	First extract.	Second extract.		
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
F. O. Woodruff, New York	0.285	0.59	0.875	1.14
G. M. Bartlett, Boston, Mass.	[a] 1.15	[a] .35	1.50	[a] 1.78
	.889	.279	1.17	1.21
A. G. Woodman, Boston, Mass.	.53	.29	.82	[a] 1.74
				1.02

[a] Gravimetric determination.

Cooperative results on lactose and sucrose in milk chocolate (Dubois method).

Analyst.	Sucrose.	Lactose.
	Per cent.	*Per cent.*
W. L. Dubois, Buffalo, N. Y.	36.90	9.10
G. M. Bartlett, Boston, Mass.	23.92	10.53
R. W. Hilts, Philadelphia, Pa.	37.05	7.32
F. O. Woodruff, New York, N. Y.	29.40	12.57
A. G. Woodman, Boston, Mass.	34.60	6.99

COMMENTS BY ANALYSTS.

G. M. Bartlett: Some difficulty was met in taking the direct reading because the end point was rather indefinite. The solution was not colored, but the transition tint was indefinite. This was not due to the polariscope and seemed only to affect the direct reading, as the invert readings were obtained without difficulty.

R. W. Hilts: Dubois's method was followed exactly as given in Bulletin 107, Revised. All solutions were adjusted to volume at 20° C. and all polarizations were made in jacketed tubes at exact temperatures of 20° and 87°.

For the sake of comparison and as a check, I also determined the lactose gravimetrically in this sample and then figured the sucrose from the polarization. In general the method followed was that suggested in the Schweizerisches Lebensmittelbuch III, page 88. Five grams of sample were defatted as in Dubois's method, extracted with warm 70 per cent alcohol, digested with the same for some time, and finally cooled and made up to 250 cc. This was filtered and the alcohol evaporated out of 200 cc of the filtrate, which was then taken up in water, clarified as usual, and made up to 200 cc. The lactose determination was made as usual by following Walker's method. Sucrose was calculated by taking the average corrected direct polarization, made by the Dubois method, and subtracting from this the rotation corresponding to the amount of lactose found gravimetrically. Results found by this method were as follows: Sucrose, 37.55 per cent by direct polarization; lactose, 7.55 per cent by Walker's gravimetric method.

I think this agreement very satisfactory, everything considered. The Dubois method is a very useful one for general and routine examinations but certainly can not lay much claim to accuracy, at least as regards lactose. This is shown by the fact that under the conditions of the method, when polarizing a fourth-normal solution at 87°, an error of 0.1° V means an error of 0.6 per cent of lactose. When greater certainty and accuracy is desired I should certainly wish to check the result by a gravimetric determination as above. This having been done, it is easy to get the sucrose from the direct polarization, and it seems reasonable to suppose that the result so obtained is more accurate than that obtained by the Clerget formula, which involves an inversion in the presence of lactose.

Recommendations.

There seems to be a considerable unanimity of opinion among those collaborating on the caffein determination that as far as regards rapidity and convenience, the Gorter method is much superior to the present provisional method.

It is conclusively shown, as has been pointed out before, that in the latter the extraction with chloroform, as directed, even prolonged for twenty hours, does not remove all the caffein, a second extraction of the moistened residue invariably yielding more caffein. It is recommended, therefore, that the Gorter method be adopted as a provisional method of the association.

The results on the determination of sugars in milk chocolate were quite disappointing. The extreme variation seems to be due principally to errors in the determination of the lactose, and it is recommended that the referee for next year on cocoa products be directed to make a critical study of the Dubois method.

DETERMINATION OF STARCH IN COCOA PRODUCTS.

By W. L. Dubois.

In an article read before the association last year the writer spoke of the tedious process of extracting fat from cocoa samples prior to hydrolysis as laid down in the provisional method and gave a few figures to support the more rapid method of extracting the fat by shaking with ether or gasoline and throwing down in the centrifuge. As stated then, this procedure removes practically all of the fat in a very short time and prepares the sample for the next operation, the process requiring about an hour for two treatments with the solvent, while the provisional method consumes sometimes a day or more. The latter is furthermore open to the objection of offering a constant opportunity for loss as the solvent climbs up the sides of the mortar. Since presenting this matter to the association the writer has worked further along the same line and finds that the most convenient container in which to treat the sample

with ether is a straight-sided vial holding about 1.5 ounces and fitted with a cork. Two grams of material can be conveniently extracted, and the residue is much more easily washed out than from a bottle with a contracted top. The sample is weighed into the bottle, and about 30 cc of ether are added and stirred thoroughly. It is then whirled in the centrifuge until clear and the ether drawn off with a small tube connected with a vacuum pump, or decanted quickly from the residue. It was found that two treatments with ether removed from 85 to 90 per cent of the fat. As stated in the previous article and supported by analytical figures, this procedure gives results as accurate as those obtained by the provisional method and with the saving of considerable time.

For the determination of starch in cocoa products, however, it has been found unnecessary to remove the fat previous to hydrolysis. In order to demonstrate this the writer extracted the fat from a cocoa of first quality by the method just given and determined the starch in the residue by the provisional method, obtaining 24.42 per cent. To 3 grams of the defatted cocoa 1 gram of cocoa butter was added and heated until thoroughly mixed; 24.65 per cent of starch was found in this mixture. A blank run on the cocoa butter gave no copper-reducing material. The starch was then determined in a number of cocoas and chocolates both before and after extracting the fat by the method just described, using 4 grams of the sample. The results given in the following table show that the presence of fat has no influence on the starch determination, and it is, therefore, unnecessary to remove it before treatment with hydrochloric acid. This makes it possible to still further shorten the method for the determination of starch in cocoa products, the sample of 4 grams being weighed directly into a 500 cc flask and hydrolysis conducted as laid down by the provisional method, except that clarification with lead acetate is omitted, this having been found to be unnecessary.

An article[a] has recently appeared describing a rapid method of hydrolyzing starch by the use of concentrated sulphuric acid. We have applied this to cocoa products with good results, finding that a reliable determination in cocoa or chocolate can be made in a fraction of the time required by the provisional method, even when modified as described above. This procedure is as follows:

Two grams of the sample are transferred to a 500 cc Erlenmeyer flask, 20 cc of water added, and then 12 cc of concentrated sulphuric acid, the latter cautiously and with slow rotation of the flask. The mixture is heated over a low flame with constant rotation until the color changes from brown to reddish black. (The time required for this change has been found to be approximately one and one-fourth minutes, so that now all samples are heated for that length of time.) Thirty cubic centimeters of water are then added, the mixture is heated to boiling, and boiled for fifteen seconds. A little cold water is poured in, the flask quickly cooled, and the acid nearly neutralized with a saturated solution of caustic potash. The solution is then again cooled and transferred to a 250 cc flask, completing to volume with cold water. Fifty cubic centimeters of the filtrate are used for the determination of copper-reducing substance as dextrose.

In this method there is no clarification with basic lead acetate and a comparison of its results with those obtained by the provisional method, in which such clarification is made (see table), shows the same to be unnecessary. By the procedure just described the hydrolysis can be accomplished in two minutes and the complete determination of starch in forty-five minutes.

The methods described are, of course, applicable to unsweetened goods only, and investigations are under way for the purpose of adapting the same to cocoa products containing sugar in varying amounts.

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[a] J. Ind. Eng. Chem., 1909, 1: 445.

Determination of starch in unsweetened cocoa products.

Sample.	Modified provisional method.		Short method.	Sample.	Modified provisional method.		Short method.
	Fat extracted.	Fat not extracted.			Fat extracted.	Fat not extracted.	
Cocoas:	*Per cent.*	*Per cent.*	*Per cent.*	Chocolates:	*Per cent.*	*Per cent.*	*Per cent.*
1	18.26	18.25	18.75 18.74	1	12.12	12.39	11.80
2	19.10	18.96	18.36 18.48	2	10.92	10.97	11.20
3	18.48	17.62	17.24 17.88	3	12.77	12.50	.....
4	16.95	16.73	17.88 17.88	4	11.24	11.32	11.95
5	19.07	18.65	18.92 18.88	5	10.63	10.74	11.38
6	19.02	19.49	21.15 21.21	6	10.10	10.48	10.99
7	18.37	18.68	18.00	7	.....	19.95	20.10
8	19.85	19.77	19.86	8	.....	19.40	20.19
9	16.31	15.57	.....	9	.....	18.00	19.05
				10	.....	17.04	17.78
				11	.....	18.21	18.86

REPORT OF COMMITTEE ON NOMINATIONS.

The chairman of the committee on nominations, Mr. B. B. Ross, presented its report, as follows: For president, Mr. W. A. Withers; for vice-president, Mr. F. W. Woll; for secretary, Mr. H. W. Wiley; for additional members of the executive committee, Mr. James M. Bartlett and Mr. James T. Willard.

The unanimous vote of the association was cast for the officers named.

REPORT ON PRESERVATIVES.

By P. B. Dunbar, *Associate Referee.*

Determination of Sodium Benzoate in Ketchup.

The work this year has been confined principally to a study of methods for the determination of sodium benzoate in ketchup. All of these methods depend upon the extraction of benzoic acid by means of immiscible solvents.

The work of last year [a] demonstrated that of the more common organic solvents, ether and chloroform are preferable for the extraction of benzoic acid. By far the most complete extraction can be obtained by means of ether; but this substance is objectionable because of its property of dissolving water and its consequent tendency to extract tannin, salts, mineral acids, and other interfering substances. The use of ether is also a source of danger because of its inflammability.

Chloroform stands next to ether in its power of dissolving benzoic acid and it has been shown that a practically complete extraction can be obtained by

[a] U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 68.

this solvent when the solution to be extracted has previously been saturated with sodium chlorid. The use of chloroform has, therefore, been recommended in all of the methods sent out by the referee this year for this reason and also because it is not inflammable and dissolves only traces of mineral acids, tannin, salts, and most of the other interfering substances. In addition to this, it is heavier than water and can be very conveniently drawn off from the bottom of the separatory funnel. The tendency to form emulsions is probably somewhat greater with chloroform than with ether, but by the use of the proper precautions this objection can be easily overcome.

On April 26, 1909, the outlines of three methods for the determination of benzoate of soda in ketchup were sent to the various collaborators with the request that they be studied. In order to secure uniformity, a sample of sodium benzoate (Merck) was sent with the methods. Pure sodium benzoate should contain one molecule of water of crystallization. All of the sodium benzoate which has been examined in this laboratory, however, has been found to be incompletely crystallized and to contain less water of crystallization than should theoretically be present.

A large number of determinations of the purity of this salt were made by extraction of known weights of the substance from water solution, both with ether and chloroform after acidification with sulphuric acid. The salt was also burned and the alkalinity of the residual sodium carbonate determined by titration with standard acid. The average of a large number of closely agreeing results showed the substance to contain 94.60 per cent of anhydrous sodium benzoate instead of 88.88 per cent, as should be the case if it contained one molecule of water of crystallization. The collaborators were requested to dissolve weighed amounts of this sodium benzoate in a small quantity of water and make up to a known weight with a ketchup which was free from sodium benzoate. The ketchups, as prepared, were not to contain more than 0.3 per cent of sodium benzoate. The methods sent out to the collaborators were as follows:

METHOD 1.

Dilute 150 grams of ketchup with about 150 cc of fresh water, make slightly alkaline to litmus paper with sodium hydroxid, and complete the dilution to 500 cc with water. Allow the mixture to stand several hours with frequent shaking, and filter through a folded filter. Transfer 150 cc of the filtrate, corresponding to 45 grams of ketchup, to a separatory funnel. (With this dilution there is usually no trouble in obtaining 300 cc of the filtrate. If necessary the filter and its contents may be squeezed through a muslin bag to remove the greater part of the solid matter and the resulting liquid filtered through a fresh folded filter.) Add 45 grams of pulverized sodium chlorid to the solution in the separatory funnel and shake until dissolved. Neutralize the solution to litmus paper with sulphuric acid (1:5) and add an excess of 5 cc of the same acid. Extract with chloroform, using successive portions of 50, 30, 25, and 25 cc.

If an emulsion forms it should be broken up by centrifuging. After centrifuging, the bubbles in the emulsion may often be further broken up by stirring with a glass rod. Since this is a progressive extraction very great care must be taken to draw off as much of the clear chloroform solution as possible after each extraction, but under no circumstances must any of the emulsion be drawn off with the chloroform.

Evaporate the combined chloroform extracts to dryness at room temperature in a current of air dried over calcium chlorid. Dissolve the residue of benzoic acid in neutral alcohol, dilute somewhat with water, and titrate with tenth-normal alkali, using phenolphthalein as indicator. Calculate as C_6H_5COONa.

METHOD 2.

To 150 grams of ketchup add 15 grams of pulverized sodium chlorid to saturate the water in the sample, and about 150 cc of a saturated solution of sodium chlorid. Make slightly alkaline to litmus paper with sodium hydroxid and complete the dilution to 500 cc with the saturated salt solution. Allow to stand several hours with frequent shaking. Filter through a folded filter and transfer a 150 cc portion of the filtrate (corresponding to 45 grams of ketchup) to a separatory funnel. Neutralize the filtrate to litmus paper with sulphuric acid (1:5) and add an excess of 5 cc of this acid. Extract with chloroform, using successive portions of 50, 30, 25, and 25 cc, observing all the precautions mentioned under Method 1. Evaporate the combined extracts, dissolve in neutral alcohol, and titrate as directed above.

These two methods are modifications of the method designated as Method 2, in the report of the referee of last year,[a] which was based on the method proposed by La Wall and Bradshaw.[b] Method 2, as outlined last year, was objectionable, owing to the large amount of solution required. Aliquot portions of 500 cc of the filtrate were used, requiring 225 cc of chloroform for extraction. The results obtained by this method were, on the whole, quite satisfactory. Methods 1 and 2, as proposed this year, were designed to lessen the quantities of solution and solvent with which it is necessary to work.

METHOD 3.

Make 150 grams of ketchup slightly alkaline with sodium hydroxid and dilute to 500 cc with saturated salt solution. Allow the mixture to stand several hours with frequent shaking and filter. Neutralize 150 cc of the filtrate with sulphuric acid (1:5), add an excess of 5 cc of the acid, and extract with chloroform as in Methods 1 and 2. Evaporate the chloroform extract to dryness in a current of dry air. Treat the residue with a saturated solution of barium hydroxid [$Ba(OH)_2$], filter, and wash thoroughly with saturated barium hydroxid. Evaporate this saturated solution to a small volume at about 70°–80°, neutralize with hydrochloric acid (1:5), add an excess of 5 cc of this acid, and extract with chloroform. Evaporate the chloroform extract to dryness in a tared porcelain dish, dry overnight in a sulphuric acid desiccator, and weigh as benzoic acid. The residue may also be dissolved in neutral alcohol and titrated as in the preceding methods.

The suggestion regarding the use of barium hydroxid as a purifying agent was made by A. F. Seeker. The details of this method, however, were added by the referee and had not been previously tested in the laboratory.

The results obtained by the three methods just given are shown in Table 1. It will be observed that both Methods 1 and 2 gave fairly satisfactory results, the average recovery by Method 1, omitting a few figures which are obviously too high or too low, being 101.1 per cent, and that by Method 2, 101.0 per cent. It is apparent that where a small amount of sodium benzoate is present there is a tendency for the results to be somewhat high. This is to be expected since any mineral acid which is drawn off with small portions of the emulsion would naturally give a higher relative error where the amount of sodium benzoate present is small.

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[a] Loc. cit., p. 70.

[b] Amer. J. Pharm., 1908, **80**: 171.

TABLE 1.—*Cooperative work by three methods for the recovery of known amounts of sodium benzoate.*

[Grams per 100 grams.]

Analyst.	Anhydrous sodium benzoate.								
	Added.	Recovered.							
		Method 1.		Method 2.		Method 3.			
						By titration.		By weight.	
			Per cent.		*Per cent.*		*Per cent.*		*Per cent.*
C. P. Wilson	0.287	0.300	104.5	0.274	95.5	0.256	89.2	.....	.....
	.287	.286	99.7	.....	.....	.270	94.1	.....	.....
	.193	.198	102.6	.182	94.3	.169	87.6	.....	.....
	.193	.199	103.1	.202	104.7	.168	87.1	.....	.....
	.096	.104	[a] 108.3	.099	103.1	.100	104.2	.....	.....
	.096	.112	[a] 116.7	.100	104.2	.103	107.3	.....	.....
	.2893	.291	97.6	.297	99.6	.282	94.5	.....	.....
	.2893	.288	96.6	.294	98.6	.272	91.2	.....	.....
	.1948	.190	97.5	.199	102.1	.181	92.9	.....	.....
	.1948	.187	96.0	.193	99.1	.181	92.9	.....	.....
	.0978	.....	.....	.100	102.3	.089	91.0	.....	.....
	.0978	.092	94.1	.101	103.3	.085	86.9	.....	.....
Fred S. Dunks	.1000	.0961	96.1	.0993	99.3	.....	.....	.....	.....
	.1000	.0993	99.3	.1008	100.8	.....	.....	.....	.....
	.2000	.1985	99.3	.2049	102.5	.....	.....	.....	.....
	.2000	.2049	102.5	.2049	102.5	.....	.....	.....	.....
	.3000	.3042	101.4	.3009	100.3	.....	.....	.....	.....
	.3000	.3042	101.4	.2994	99.8	.....	.....	.....	.....
T. F. Pappe	.0955	.0949	99.4	.0959	100.4	.....	.....	.....	.....
	.0955	.0923	96.7	.0929	97.3	.....	.....	.....	.....
	.2845	.2740	96.3	.2725	95.8	.....	.....	.....	.....
	.2845	.2693	94.7	.2701	94.9	.....	.....	.....	.....
G. R. Stewart	.1500	.1566	104.4	.1551	103.4	.143	95.4	0.1500	100.0
	.1500	.1573	104.8	.1584	105.6	.142	94.6	.1495	99.7
	.1500	.1568	104.6	.1513	100.8	.....	.....	.....	.....
	.1500	.1568	104.6	.1504	100.2	.....	.....	.....	.....
	.2000	.2124	106.2	.2064	103.2	.1954	97.7	.2037	101.9
	.2000	.2111	105.6	.2095	104.7	.1920	96.0	.2022	101.1
	.2000	.2111	105.6	.2080	104.0	.....	.....	.....	.....
	.2000	.2095	104.8	.2064	103.2	.....	.....	.....	.....
	.3000	.3104	103.5	.3200	106.7	.2896	96.5	.2982	99.4
	.3000	.3133	104.4	.3200	106.7	.2922	97.4	.2958	98.6
	.3000	.3007	100.2	.2975	99.2	.....	.....	.....	.....
	.3000	.3055	101.8	.2991	99.7	.....	.....	.....	.....
D. B. Bisbee	.250	.260	104.0	.253	101.4	.....	.....	.241	96.4
R. W. Hilts	.191	.206	107.8	.197	103.1	.194	101.5	.208	108.9
	.191	.202	105.7	.....	.....	.....	.....	.....	.....
	.102	.....	.....	.106	103.9	.....	.....	.....	.....
C. Conover	.1764	.1791	101.5	.1791	101.5	.1504	85.3	.1600	90.7
	.1764	.....	.....	.1776	100.6	.....	.....	.....	.....
A. L. Knisely	.2500	.256	102.4	.2500	100.0	.2240	89.6	.2373	94.9
	.2500	.257	102.8	.2590	103.6	.2310	92.4	.2303	92.1
A. E. Taylor	.1892	.2016	106.6	.1953	103.2	.1800	95.1	.....	.....
W. C. Burnet	.1892	.1922	101.6	.2082	[a] 110.0	.1831	96.7	.....	.....
H. L. Jackson	.3337	.3852	[a] 115.4	.....	.....	.....	.....	.....	.....
	.3337	.3248	97.3	.....	.....	.....	.....	.....	.....
	.2499	.....	.....	.2505	100.2	.....	.....	.....	.....
	.2499	.....	.....	.2358	94.4	.....	.....	.....	.....
	.3445	.....	.....	.3446	100.0	.....	.....	.....	.....
	.3445	.....	.....	.3324	96.5	.....	.....	.....	.....
	.2722	.....	.....	.2880	105.8	.....	.....	.....	.....
	.2722	.....	.....	.2999	[a] 111.0	.....	.....	.....	.....
A. L. Davison	.1854	.1857	100.1	.....	.....	.....	.....	.....	.....
	.1854	.1793	96.9	.....	.....	.....	.....	.....	.....
	.1930	.1975	102.3	.....	.....	.....	.....	.....	.....
	.1930	.1956	101.3	.....	.....	.....	.....	.....	.....
	.1933	.....	.....	.1969	101.8	.....	.....	.....	.....
	.1073	.....	.....	.1124	104.7	.....	.....	.....	.....
	.1073	.....	.....	.1057	98.5	.....	.....	.....	.....
J. M. Bartlett	.189	.174	[a] 92.1	.195	103.2	.171	90.5	.182	96.3
F. F. Flanders	.183	.192	104.9	.180	98.4	.171	93.4	.175	95.6
	.183	.192	104.9	.180	98.4	.180	98.4	.182	99.4
N. Hendrickson	.0971	.0936	96.4	.....	.....	.....	.....	.....	.....
	.0971	.0936	96.4	.....	.....	.....	.....	.....	.....
	.0966	.0913	94.5	.....	.....	.....	.....	.....	.....
	.0966	.0928	96.1	.....	.....	.....	.....	.....	.....
	.2473	.....	.....	.2474	100.0	.....	.....	.....	.....
	.0826	.....	.....	.0897	[a] 108.6	.....	.....	.....	.....
	.0826	.....	.....	.0912	[a] 110.4	.....	.....	.....	.....

[a] Not included in average.

TABLE 1.—*Cooperative work by three methods for the recovery of known amounts of sodium benzoate*—Continued.

Analyst.	Anhydrous sodium benzoate.								
	Added.	Recovered.							
		Method 1.		Method 2.		Method 3.			
						By titration.		By weight.	
			Per cent.		*Per cent.*		*Per cent.*		*Per cent.*
P. B. Dunbar	0.2816	0.2831	100.5	0.2739	97.2	0.2002	70.7	0.2133	75.7
	.2816	.2840	100.9	.2773	98.5	.2413	85.2	.2539	90.2
	.2064	.2093	101.4	.2162	104.7	.1680	81.4	.1852	89.7
	.2064	.....	.....	.2113	102.4	.....	.....	.....	.....
	.1133	.1176	103.8	.1193	105.4	.0829	73.2	.0900	79.4
	.1133	.1162	104.6	.1133	100.0	.0820	72.4	.0944	83.3
C. A. Brautlecht	.250	.250	100.0	.246	98.4	.....	.....	.....	.....
	.250	.253	101.2	.246	98.4	.....	.....	.....	.....
E. M. Bailey	.250	.253	101.2	.256	102.4	.....	.....	.....	.....
	.250	.253	101.2	.250	100.0	.....	.....	.....	.....
Average	.....	.....	101.1	.....	101.0	.....	.....	.....	.....

COMMENTS OF ANALYSTS.

The majority of collaborators agreed that of these three methods, No. 2 was much to be preferred. Method 1 was considered objectionable owing to the fact that the addition of salt after filtration often caused a precipitate which gave rise to a very troublesome emulsion on extraction with chloroform. Method 3 was found to be quite tedious and the results were usually low.

H. L. Jackson: I do not like Method 1, as the addition of salt to the clear filtrate in the separatory funnel gave a further precipitate, which caused a very troublesome emulsion to form. The high results are probably all due to sulphuric acid going through, though I was not aware of including any of the emulsion. In the future I would draw off the chloroform into a second separatory funnel containing clean water and thus wash out the sulphuric acid. On testing for sulphates once they were found present, and on titrating a couple of times with tenth-normal barium hydroxid there was a slight precipitate. I think Method 2 can be made to work well.

R. W. Hilts: In all cases the mass was squeezed through a linen bag and run through two thicknesses of cheese cloth before filtering through paper. No difficulty was experienced in obtaining over 300 cc of filtrate. No especial difficulty was found in making the extraction; funnels were agitated thoroughly but not violently for two minutes each time. The emulsions were broken by placing funnels in centrifuge, by which means very fair separations were obtained. Without the centrifuge the emulsions would have presented considerable difficulty. Of the methods of extraction given, Method 2 seems preferable, as the filtration is more rapid, the filtrate is cleaner, there is less tendency to form obstinate emulsions, and the benzoic acid residue is somewhat purer than by the first method.

G. R. Stewart: In titrating with Methods 1 and 2 the end point is not very definite, owing to the slightly colored solution, and in any case the error of titration is quite large considering the number of times it is multiplied in the final result. We prefer a method in which the residue is purified and weighed as in Method 3. The final result obtained by this method was quite clean, almost perfectly white, and the results agree very closely with that which was added.

C. Conover: Method 2 was preferable as far as the filtration was concerned. By Methods 1 and 2 over 100 per cent of sodium benzoate was obtained. I would suggest that this error is due to the use of sulphuric acid and that hydrochloric acid should be used instead.

T. F. Pappe: While these results show that practically all of the benzoic acid is recovered in both methods, I am not satisfied to report this as benzoic

acid, inasmuch as a blank run along with the analysis in both methods gives distinct titration with alkali. While it may be that in most cases this error due to acid extractives in the ketchup would counterbalance the error due to incomplete extraction of benzoic acid, still I do not believe this would be a safe assumption to make. With Method 3 I have obtained no results which I would consider satisfactory to report. I find that on adding salt to my filtrate in Method 1 a certain amount of soluble matter is thrown out of solution which tends to emulsify on shaking with chloroform. I consider Method 2 the best on account of the greater ease of manipulation. When the salt is added to the ketchup and the sample made up to 500 cc with saturated salt solution, as in this method, the solution filters much more rapidly and the filtrate obtained is much clearer than in Method 1.

Fred S. Dunks: I would suggest that twentieth-normal sodium hydroxid be used in place of tenth-normal sodium hydroxid, as more accurate results could be obtained.

C. P. Wilson: These results, I think, demonstrate that Method 2 is the best. It is the simplest to use and gives the least trouble and chance for error. I do not like Method 3, as it is very long and tedious and is almost sure to give low results.

D. B. Bisbee: Method 2 gave a more easily extracted solution, and seemed to work better all the way through. Method 3 is longer and there is an opportunity for loss of benzoate from one end of the process to the other. On account of the additional time necessary, to say nothing of the additional cost of chemicals used, it is my opinion that Method 3 is not as satisfactory as Method 2. In all cases I used hydrochloric acid in acidifying the ketchup solution before extraction in order to prevent, as far as possible, error due to mechanically carried through acid from the solution being extracted.

J. M. Bartlett: Method 2 was considered much the easiest, while Method 3 was very tedious and impracticable, although the results obtained were quite good.

As a result of the reports just given and the almost unanimous preference expressed by the collaborators for Method 2, the method was modified so as to include many of the suggestions which were made by the collaborators and sent out to the collaborators on June 26, together with three bottles of ketchup containing known amounts of benzoate of soda.

The method as revised and sent out was practically as follows, though further variations have been made as a result of additional suggestions by the collaborators:

METHOD 4 (METHOD 2 REVISED).

To 150 grams of ketchup add 15 grams of pulverized sodium chlorid to saturate the water in the ketchup. Transfer the mixture to a 500 cc graduated flask, using about 150 cc of a saturated solution of sodium chlorid for rinsing. Make slightly alkaline to litmus paper with strong sodium hydroxid solution and complete the dilution to 500 cc with the saturated salt solution. Allow to stand at least two hours with frequent shaking. Squeeze through a heavy muslin bag and then filter through a large folded filter. Enough solution will be obtained for duplicate determinations. Pipette 150 cc of the filtrate into a separatory funnel. Neutralize the solution to litmus paper with sulphuric or hydrochloric acid (1:5) and add an excess of 5 cc of the same acid. Extract carefully with chloroform, using successive portions of 70, 50, 40, and 30 cc, drawing off the chloroform into a second separatory funnel after each extraction. (Note 1.) Wash the combined extract with two 15 cc portions of distilled water, taking care not to lose any of the chloroform when pouring off the wash water. Transfer the washed extract to a porcelain evaporating dish, rinsing the separatory funnel three times with 5 or 10 cc portions of chloroform and evaporate to dryness in a current of dry air. (Note 2.) Dry the residue overnight, or until no odor of acetic acid can be detected, in a sulphuric acid desiccator. Dissolve the residue of benzoic acid in neutral alcohol (30–50 cc), add about one-fourth this volume of water and a drop or two of phenolphthalein solution, and titrate with twentieth-normal sodium hydroxid. Calculate the results as anhydrous sodium benzoate; 1 cc of twentieth-normal sodium hydroxid=0.0072 gram of anhydrous sodium benzoate.

NOTE 1.—To avoid emulsion, shake each time cautiously for about a minute. Vigorous shaking is not necessary. The chloroform layer usually separates

readily at the bottom of the funnel after standing a few minutes. If any emulsion forms it can often be broken up by stirring the chloroform layer with a glass rod. If this is unsuccessful, the emulsified portion may be drawn off into a second funnel and given one or two sharp shakes from one end of the funnel to the other. If this also fails, the emulsion should be centrifuged for a few minutes. The funnels should be carefully rinsed out with chloroform after each of these operations.

As this is a progressive extraction, very great care must be taken to draw off as much of the clear chloroform solution as possible after each extraction, but under no circumstances must any of the emulsion be drawn off with the chloroform.

NOTE 2.—If a blast is convenient, it is preferable to evaporate the whole extract at room temperature. For this purpose the following simple apparatus is convenient: A wide mouth salt bottle is fitted with a cork; a glass tube extends through the center of the cork to the bottom of the bottle, and its upper end is attached to the blast by a rubber tube. As many other glass tubes as convenient are passed through the cork around the central tube. These terminate just inside the cork, and outside the cork are bent outward and downward at an angle of about 45°. The bottle is filled with calcium chlorid and by this means a current of dry air can be delivered to the dish containing the extract. In the absence of a blast an electric fan may be used for evaporating the extract.

If it is impracticable to evaporate the chloroform spontaneously or by means of a blast, it may be transferred from the separatory funnel to a 300 cc Erlenmeyer flask, rinsing the separatory funnel three times with 5 or 10 cc of chloroform. Distil very carefully to about one-fourth the original volume, keeping the temperature down so that the chloroform comes over in drops, not in a steady stream. Then transfer the extract to a porcelain evaporating dish, rinsing the flask three times with 5 or 10 cc portions of chloroform, and evaporate to dryness spontaneously.

TABLE 2.—*Cooperative results on determination of sodium benzoate by Method 4 (2 revised).*

Analyst.	Anhydrous sodium benzoate.			Analyst.	Anhydrous sodium benzoate.		
	Added.	Recovered.			Added.	Recovered.	
	Grams per 100 grams.	Grams per 100 grams.	Per cent.		Grams per 100 grams.	Grams per 100 grams.	Per cent.
T. F. Pappe	0.0934	0.0755	80.8	W. A. Bender	0.1113	0.075	67.4
	.0934	.0770	82.4		.1113	.070	62.9
	.2207	.1980	89.7		.2015	.200	99.3
	.2207	.2024	91.7		.2015	.205	101.7
	.3083	.2852	92.5		.2867	.286	99.8
	.3083	.2845	92.3		.2867	.287	100.1
C. Conover	.0934	.074	79.2	A. E. Taylor	.1113	.0791	71.1
	.0934	.074	79.2		.1113	.0727	65.4
	.2207	.194	87.9		.1113	.0887	79.7
	.2207	.200	90.6		.2015	.1537	76.3
	.3083	.278	90.2		.2015	.1656	82.2
	.3083	.272	88.2		.2015	.1762	87.4
R. W. Hilts	.0934	.091	97.4		.2867	.2556	89.2
	.0934	.091	97.4		.2867	.2530	88.3
	.2207	.209	94.6		.2867	.2594	90.5
	.2207	.211	95.5	H. L. Jackson	.1113	.101	90.7
	.3083	.296	96.1		.1113	.097	87.1
	.3083	.297	96.4		.2015	.151	74.9
A. L. Davison	.1113	.1240	111.4		.2867	.243	84.8
	.1113	.1248	112.1		.2867	.238	83.0
	.2015	.2008	99.7	N. Hendrickson	.1055	.100	95.2
	.2015	.2008	99.7		.1055	.1005	95.7
	.2867	.3000	104.6		.2365	.2192	92.7
	.2867	.2944	102.7		.2365	.2213	93.6
A. L. Knisely	.1113	.1152	103.5		.2365	.2241	94.7
	.1113	.1120	100.6		.3143	.2706	86.1
	.2015	.1952	96.9		.3143	.2800	89.1
	.2015	.2048	101.6				
	.2867	.2752	96.0				
	.2867	.2784	97.1				

COMMENTS OF ANALYSTS.

T. F. Pappe: The washed chloroform was distilled from approximately 200 cc to approximately 60 cc at a very low temperature. I do not feel that this procedure is advantageous and would recommend that the chloroform be allowed to evaporate spontaneously unless a blast is at hand.

A. L. Kniscly: I experienced no difficulty whatever in any part of the method.

H. L. Jackson: I was always a little uncertain as to the end point and used a touch plate in making the titrations.

R. W. Hilts: To evaporate chloroform at room temperature, it is very convenient to set the beakers in a pan of water on the electric hot plate set at the lowest heat, maintaining the water at 40°. The dried air current is blown into the beakers and the chloroform evaporates at a fair speed at a temperature of 30° or below. There is no tendency for water to condense in the beakers.

A number of determinations have been made by the following method, which is based on Mr. Seeker's suggestion that barium hydroxid be used in purifying the benzoic acid residue. The details of this method were worked out by G. R. Stewart.

METHOD 5 (SEEKER METHOD).

Place 100 grams of ketchup in a 200 cc flask, make slightly alkaline with sodium hydroxid, make up to volume, shake thoroughly and filter through a dry filter; acidify either 75 or 100 cc of filtrate with 5 cc of (1:5) sulphuric acid and shake out with 100, 75, 50, and 50 cc portions of ether. Combine the ether extracts and distil off most of the solvent leaving 10–20 cc in the flask. Transfer this residue to a dish or capsule and rinse out the flask with a little ether, adding the rinsings to the dish. Evaporate to dryness spontaneously and take up the residue with about 20 cc of saturated barium hydroxid. Filter through a small wetted filter and wash thoroughly with barium hydroxid, for which about five portions of 10 cc each are necessary. Evaporate the filtrate on a steam bath to a volume of about 30–40 cc, neutralize with hydrochloric acid and add an excess of 2 cc of concentrated hydrochloric acid. Shake out with ether, using three successive portions, each portion equal in volume to that of the aqueous layer, wash the combined ether extracts with two 5 cc portions of distilled water, and evaporate the ether extract to dryness in a tared dish, drying to constant weight in a desiccator over sulphuric acid. The residue of benzoic acid may also be titrated.

TABLE 3.—*Determination of sodium benzoate by Method 5 (Seeker).*

[Grams per 100 grams.]

Analyst.	Anhydrous sodium benzoate.				
	Added.	Recovered.			
		By weight.		By titration.	
			Per cent.		*Per cent.*
G. R. Stewart	0.15	0.1383	92.2	0.1354	90.3
	.20	.1897	94.9	.1830	91.5
	.30	.2922	97.4	.2852	95.0
C. P. Wilson	.287	.....	.....	.2512	87.5
	.287	.....	.....	.2203	76.8
	.193	.....	.....	.1637	84.8
	.193	.....	.....	.1787	92.6
	.096	.....	.....	.0931	97.0
	.096	.....	.....	.0888	92.5
	.2893	.....	.....	.285	95.5
	.1948	.....	.....	.187	96.0
	.0978	.....	.....	.101	103.3

A method proposed by A. E. Taylor, of the Savannah laboratory, has given very satisfactory results.

METHOD 6 (TAYLOR METHOD).

Transfer 150 grams to a 500-cc flask with about 150 cc of distilled water; make alkaline to litmus paper with saturated barium hydroxid solution, shaking well; then add 10 to 15 cc excess of barium hydroxid. Make to the mark with water and let stand two hours, shaking frequently. Squeeze through heavy muslin; then filter through a large folded filter paper. Enough solution will be obtained for duplicate determinations. Pipette 150 cc of the solution into a separatory funnel, add 5 cc of saturated barium hydroxid, and extract with 50 cc of washed ether. (See Note 1.) Discard the ether after washing it two or three times with 5 cc portions of water. (See Note 2.) Add the washings to the aqueous solution and neutralize with hydrochloric acid (1 to 3), adding 5 cc excess of the same acid. Extract with 70 cc, 50 cc, 40 cc, and 40 cc portions of washed ether. After separating from the aqueous solution, wash each ether portion but the last, two or three times with 5 cc of distilled water, returning the washings to the aqueous solution. Wash the combined ether extract in the same way with two or three portions of water, using 5 cc each time.

Transfer the combined ether extract to a 300-cc Erlenmeyer flask; rinse the separatory funnel three times with ether from a pipette, using 5 or 10 cc each time. Distil very carefully (Note 3) to about one-fourth the original volume; then transfer the ether to a weighed evaporating dish, rinsing the flask three times with 5 cc portions of ether. Evaporate spontaneously in the sun or in a current of dry air; then dry in a desiccator containing sulphuric acid overnight or until no odor of acetic acid can be detected (Note 4); weigh (Note 5). Dissolve the benzoic-acid residue in neutral alcohol; add a drop of phenolphthalein and titrate slowly with twentieth-normal sodium hydroxid, stirring constantly. A little water (about 10 or 15 cc) may be added toward the end of the titration. Calculate the results as anhydrous sodium benzoate.

One cc twentieth-normal sodium hydroxid=0.0072 gram of anhydrous sodium benzoate.

Of course the usual care in separating the ether and the aqueous portion must be observed, as well as the usual precaution of rinsing out the funnels, after the ether extract has been run out, with the portion of ether to be used in the next extraction.

NOTE 1.—Ether washed four times with water.

NOTE 2.—The barium benzoate is not extracted by the ether, but oils, etc., are.

NOTE 3.—Just slowly enough so that the ether comes over in drops; not in a steady stream.

NOTE 4.—This will give practically constant weight.

NOTE 5.—This weight is valuable only as a check and should not be over 5 to 7 mg greater than the weight given by titration.

TABLE 4.—*Determination of sodium benzoate by Method 6 (Taylor method).*

[Grams per 100 grams.]

Analyst.	Anhydrous sodium benzoate.				
	Added.	Recovered.			
		By weight.		By titration.	
			Per cent.		*Per cent.*
A. L. Davison	0.0976	0.1080	110.6	0.0961	98.4
	.0976	.1074	109.9	.0929	95.1
	.1356	.1496	110.3	.1361	100.3
	.1356	.1399	103.1	.1377	101.5
C. Conover	.1316	.1382	105.1	.1296	98.5
	.1316	.1442	109.6	.1296	98.5

TABLE 4.—*Determination of sodium benzoate by Method 6 (Taylor method)*—Continued.

Analyst.	Anhydrous sodium benzoate.				
	Added.	Recovered.			
		By weight.		By titration.	
			Per cent.		*Per cent.*
A. E. Taylor	[a]0.1113			0.1121	100.7
	[a].1113			.1130	101.5
	[a].2015			.2017	100.1
	[a].2015			.1905	94.5
	[a].2867			.2731	95.3
	[a].2867			.2760	96.3
	[b].1892	0.1944	102.7	.1729	91.4
	[b].1892	.1959	103.5	.1849	97.7
	.1892	.2073	109.5	.1904	100.6
	.1892	.2025	107.0	.1911	101.0
W. H. Scott	[b].1892	.2041	107.9	.1867	98.7
	[b].1892	.1973	104.3	.1889	99.8
	.1892	.2031	107.3	.1898	100.3
	.1892	.2101	111.0	.1922	101.6
P. B. Dunbar	.0976	.1173	120.3	.1027	105.2
	.0976	.1252	128.3	.1037	106.2
	.0976	.1241	127.3	.1056	108.2
	.0976	.1050	107.5	.0940	96.3
	.1356	.1762	129.9	.1395	102.9
	.1356	.1436	105.8	.1207	89.0

[a] Official samples, amount of preservative present not known to analyst.
[b] An excess of 5 cc of 10 per cent hydrochloric acid was added to the aqueous solution instead of 5 cc of concentrated hydrochloric acid as directed in the method.

COMMENTS OF ANALYSTS.

C. Conover.—This method gives very good results by titration. I think, however, that Method 2 is less fussy and requires less manipulation.

W. C. Burnet.—It was noticed that the ether extraction was slightly colored. This coloration disappears, however, on exposure to the sun. Making a preliminary alkaline extraction seems to improve the appearance of the final residue. Washing the ether to remove the alcohol appears to reduce the amount of foreign substances extracted by the ether.

By this method, the ketchup filters easily and a very clean filtrate is obtained. There is little trouble by reason of emulsions. The necessity for washing each separate portion of ether makes the method very tedious, and apparently introduces many opportunities for losing benzoic acid. The results obtained, however, have been on the whole very good.

Edmund Clark, of the Boston Food Inspection Laboratory, proposes the following method:

METHOD 7 (CLARK METHOD).

Make up 50 grams of ketchup to 200 cc, mix and filter through a folded filter. Acidify 100 cc of the filtrate with dilute hydrochloric acid (1 to 4) and extract three times with 100 cc portions of ordinary ether. Transfer the ether to a 300 cc flask, introduce glass beads, and distil the ether rapidly by an electric stove to 5 cc. Disconnect the flask and exhaust the ether by suction. Add 2 cc of 20 per cent sodium hydroxid and 15 cc of water, and wash the solution into a 200 cc Squibb separatory funnel. To the ether flask from which the transfer was made add 5 cc of dilute hydrochloric acid (1:4), and wash also into the separatory funnel, dislodging any adhering particles with the aid of a glass rod. Keep the solution in the separatory down to about 50 cc. Shake out with chloroform four times, using 40, 30, 20, and 10 cc in succession. The first 40 cc may be used to rinse the flask. Run the chloroform into another 200 cc separatory funnel and wash with 30 cc of water. Run the washed extract into a 300 cc separatory funnel, add 100 cc of boiled distilled water, a few drops of phenolphthalein, and titrate immediately with twentieth-normal sodium hydroxid.

According to the author, the advantages of this method are: First, the short time required for complete analysis, namely, one hour and forty-five minutes; second, loss of benzoic acid reduced to a minimum, dependent upon the number of extractions made with the solvent; third, high results avoided by elimination of other ether extracted organic solvents, such as tartaric, succinic, malic, tannic, citric, and oxalic acids, which are insoluble in chloroform. Acetic acid, which appears to be somewhat soluble in chloroform, and hydrochloric acid mechanically present, are easily washed from the chloroform without loss of benzoic acid; fourth, identification of benzoic acid may be accomplished as easily as in any other method; fifth, economy of operation by saving in time and recovery of solvents. The results obtained by this method are given in Table 5.

TABLE 5.—*Determination of sodium benzoate by Method 7 (Clark method).*

[Grams per 100 grams.]

Analyst.	Sodium benzoate.			Remarks.
	Added.	Recovered.		
			Per cent.	
Edmund Clark	0.2473	0.228	92.2	Amount of preservative unknown to analyst; not corrected for incomplete crystallization.
	.2473	.236	95.4	Do.
N. Hendrickson	.2473	.2339	94.6	Do.
	.2473	.2419	97.8	Do.
	.2473	.2283	92.3	Do.
	.2473	.2506	101.3	Do.
	.1682	.167	99.3	Do.
	.1682	.1615	96.0	Do.
	.1682	.1556	92.5	Do.
	.1076	.1086	100.9	Not corrected for incomplete crystallization.
	.1038	.1113	107.2	Do.
	.1007	.0922	91.6	Do.
	.1057	.0974	92.1	Do.
	.2473	.2411	97.5	Do.
	.1055	.1025	97.2	Amount of preservative unknown to analyst.
	.2365	.2102	88.9	Do.
	.3143	.2678	85.2	Do.

DETERMINATION OF SODIUM BENZOATE IN CIDER.

A few experiments were made on the determination of sodium benzoate in hard cider. The following method was employed:

Make 250 cc of cider slightly alkaline to litmus paper with sodium hydroxid, and evaporate on the steam bath to about 100 cc to remove the alcohol. Then add 30 grams of salt and make up the solution to the original volume (250 cc) with saturated salt solution. Allow the mixture to stand several hours with occasional shaking, and then filter through a folded filter. Acidify 100 cc portions of the filtrate to litmus paper with 1 to 5 sulphuric acid and add an excess of 5 cc of the same acid. Extract the solution with chloroform using 50, 40, 30, and 25 cc portions. The subsequent treatment is the same as that given in Method 4.

TABLE 6.—*Determination of sodium benzoate in hard cider.*

[Grams per 100 cc.]

Anhydrous sodium benzoate.		
Added.	Recovered.	
		Per cent.
0.2066	0.2010	97.3
.2066	.1966	95.2
.2066	.2177	105.3
.2066	.2196	106.3

Determination of Sodium Benzoate in Jam.

Two methods have been tried by A. E. Taylor for the determination of sodium benzoate in jam. A mixture of peach and plum glucose jams containing a known amount of sodium benzoate was used for these determinations. The first method employed by Mr. Taylor was the same as Method 6, for the determination of sodium benzoate in ketchup, with the following exceptions: In making alkaline, the first time, 50 cc of milk of lime was substituted for barium hydroxid. The solution was finally made acid with 5 cc of sulphuric acid instead of hydrochloric acid. In each ether extraction, and in the combined extracts, three washings with water were made, using 3 cc for each washing. In the second method, the same amounts of material were used as in the previous method. After transferring to a 500-cc flask, the contents were made slightly alkaline with dilute sodium hydroxid, 15 or 20 cc of lead subacetate were added and, after shaking, about 15 or 20 cc of saturated salt solution. Thereafter the process was the same as in the previous method. No alkaline extraction was made, however, and 5 cc of 10 per cent hydrochloric acid were added to the portion used for the extraction. These methods were found to be easy of manipulation and gave no emulsions. The results obtained are given in Table 7.

Table 7.—*Determination of benzoic acid in jams.*

[Grams per 100 grams.]

Analyst.	Added.	Recovered.			
		By titration.		By weight.[a]	
			Per cent.		*Per cent.*
A. E. Taylor	0.1892	0.1962	103.7	2033	107.5
W. H. Scott	.1892	.2002	105.8	2118	111.9
A. E. Taylor	.1892	.1777	93.9	1887	99.7
W. H. Scott	.1892	.1800	95.1	2047	108.2

[a] Weighed as benzoic acid; calculated as anhydrous sodium benzoate.

Detection of Formaldehyde.

A. V. H. Mory makes the following comments on the qualitative detection of formaldehyde:

If a solution of either ethyl or methyl alcohol, before or after oxidation by means of a copper coil, be mixed with from one to two volumes of commercial sulphuric acid (66° B.) and one volume of milk be then added and the whole mixed together, a strong purple coloration results immediately, or on heating moderately. In studying this reaction the following points were observed:

(1) The presence of ferric-chlorid appeared to have no significance; equally strong coloration was produced by the plain commercial acid and that to which a small amount of ferric-chlorid had been added.

(2) Increasing the strength of the alcohol solution intensifies the color, though a strong color was obtained with a solution of only 0.1 per cent ethyl alcohol. No color was given by solutions containing 0.01 or 0.001 per cent alcohol.

(3) Preliminary heating of the alcohol-acid mixture before adding the milk intensifies the coloration resulting after the addition of the milk.

(4) The degree of dilution of the alcohol-acid mixture is also a factor. This was determined directly and is also assumed to account for the observed facts that mixing the alcohol solution and milk before adding the acid or not allowing the acid-alcohol mixture to heat up before the addition of the milk interfered greatly with the production of the color.

(5) Chemically pure sulphuric acid does not produce this reaction.

(6) The color produced as described can not be distinguished from that given in the test for formaldehyde in which the alcohol solution is omitted.

The milk used in this work gave no test for formaldehyde by Leach's test.

REPORT ON DETERMINATION OF WATER IN FOODS.

By P. F. Trowbridge, *Associate Referee.*

Methods Studied.

The work of the referee has consisted of a comparison of the official methods for moisture determinations with the alcohol method proposed by Lowenstein [a] and with the modified Benedict vacuum method without the aid of heat.[b]

At the Missouri experiment station the vacuum method has almost entirely displaced the official methods. It is not so rapid, but the results are much more reliable. Most food products undergo more or less change upon being heated for several hours at 105° even if it is done in a vacuum or in an atmosphere of hydrogen.

The alcohol method hastens the removal of the moisture and gives results in agreement with the official methods, but it is open to the same objections because of the heat employed.

The vacuum method is somewhat slower, but for all work, except factory control, can be made reasonably rapid and the results are reliable. Decomposition of the sample does not take place. The method is especially advantageous in all food materials that are to be extracted with ether after the determination of the moisture. When lean meat samples have been dried with heat the determination of the amount of the ether soluble material is a very difficult process.

Several samples were sent out to those who offered to cooperate, with the following instructions:

Instructions for Cooperative Work.

I. Official method (foods and feeding stuffs), Bul. 107, Revised, p. 38.

II. Provisional method (molasses), Bul. 107, Revised, p. 65 (3).

III. Vacuum method; (*a*) *For corn meal.* Mix the sample thoroughly. Weigh out by difference from a stoppered weighing bottle into tared crucibles about 2 grams of the sample. Tare a cover with the crucible. Place 200 cc of fresh c. p. sulphuric acid in a good 6-inch vacuum desiccator. Put triplicate samples in the desiccator, smear the edges of the latter and the stopcock with a lubricant,[b] and exhaust by means of a vacuum pump. If a pump is not at hand add 10 cc of ether to a small beaker, place in the desiccator and exhaust with a water filter pump. Use a manometer and bring the vacuum down to 5 mm or lower. Gently rotate the desiccator four or five times during the first twelve hours to mix the sulphuric acid with the water which has collected as an upper layer.

At the end of twenty-four hours open the desiccator after letting air bubbles through c. p. sulphuric acid. If a good vacuum has been maintained the samples are ready for the first weighing. After weighing, place in a desiccator with fresh c. p. sulphuric acid and exhaust as before. Rotate the desiccator once or twice and weigh again at the end of twenty-four hours, repeating until the weight is constant. Calculate the loss in weight to per cent moisture.

(*b*) *For molasses and beef extract.* Tare the crucible or flat aluminum dish with ignited quartz sand and a small stirring rod. Weigh out about 2 grams of the sample and incorporate thoroughly with plenty of sand. Proceed with the drying as directed under (*a*), making determinations in triplicate.

IV. Lowenstein's alcohol method [a] (to be used with sorghum and beef extract only).

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[a] J. Ind. Eng. Chem., 1909, 1: 252.

[b] U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 219.

Analytical Results.

Alice L. Davison, of the Division of Foods, Bureau of Chemistry, reports for the corn meal sample as follows: By the official method, 11.25, 11.23, and 11.32; by the vacuum method, 12.47, 12.56, and 12.62 per cent. C. R. Moulton, of the Missouri experiment station, finds on the same sample by the official method, 12.834 and 12.832 per cent, and by the vacuum method, 12.48 and 12.18 per cent.

In the official method a vacuum oven was used at 105° C., pressure about 25 cm. Three dryings were necessary. In the vacuum method two dryings gave the results reported, but the samples were in vacuum seventy-two hours, owing to pressure of other work. The vacuum was practically complete, less than a millimeter, obtained by the Geryk vacuum pump. On the sample of sorghum the two chemists find as follows:

Comparison of moisture determination on sorghum by different methods.

Analyst.	Official.	Vacuum.	Alcohol.
	Per cent.	*Per cent.*	*Per cent.*
A. L. Davison[a]	25.09	21.63	25.50
	25.10	21.76	25.48
	25.01	21.58	25.46
Average	25.07	21.66	25.48
C. R. Moulton	27.69	21.94	25.50
	27.85	21.54	25.95
Average	27.77	21.74	25.73

[a] By refractometer, 25.23 per cent.

In the official and alcohol methods four dryings were necessary to obtain the results reported. In the vacuum method the sample was in vacuum a total of ninety-six hours with three weighings.

In a sample of beef extract sent to Byron McClelland, of the New York Food and Drug Inspection Laboratory, 19.79 per cent of moisture is reported by the vacuum method. Mr. Moulton finds by the same method 20.44 per cent. In another sample of beef extract Mr. Moulton finds 31.37 per cent of moisture by the official method and 25.27 per cent by the vacuum method. In order to get reasonably constant weight in the official method it was necessary to make seven dryings and weighings. The third weighing by the vacuum method showed the weight to be constant. For still another beef extract, Moulton finds as follows:

Moisture results on beef extract by three methods (Moulton).

Official (four dryings).	Vacuum (three dryings).	Alcohol (four dryings).
Per cent.	*Per cent.*	*Per cent.*
32.957	27.683	32.018
33.246	27.476	31.648
Average.. 33.102	27.580	31.833

A portion of another beef extract was sent to Mr. A. Lowenstein, who made a very careful comparison of the three methods for the determination of moisture and reported as follows, Mr. Moulton also reporting on the same sample:

Moisture results by Lowenstein and Moulton on beef extract.

Analyst.	Official method.	Vacuum method.	Alcohol method.
Lowenstein	[a] 26.12, 26.40	[d] 22.30, 22.40	[e] 26.51, 26.64
	[b] 26.47	.....	.....
	[c] 26.67, 26.49, 26.25	.....	.....
Average	26.40	22.35	26.58
Moulton	[f] 25.666, 25.594	[g] 21.481, 21.343	[h] 22.855, 24.502
Average	25.63	21.412	.....

[a] Three hours in vacuum oven at 101–103° C. Pressure 11 to 16 cm. Mixed with quartz sand.
[b] Taken after drying by vacuum method and dried two hours in vacuum oven under same condition as (a).
[c] Dried without admixture of sand.
[d] Ninety-four hours in vacuum desiccator over sulphuric acid.
[e] Two portions of alcohol 15 cc each, thirty minutes on the steam bath and two hours in oven.
[f] Results in four dryings.
[g] Results in three dryings, ninety-six hours in vacuum.
[h] Results in four dryings, did not continue to constant weight.

Mr. Lowenstein also tested the methods on three commercial beef extracts with the following results:

Moisture determinations on commercial beef extracts (Lowenstein).

Description of sample.	Method.		
	Official.	Vacuum.[a]	Alcohol.
	Per cent.	*Per cent.*	*Per cent.*
(a) Low salt extract, 4.50 per cent sodium chlorid	[b] 28.98, [c] 27.38	20.62	[d] 28.40, [e] 28.92
(b) High salt extract, 18.5 per cent sodium chlorid	[b] 28.30, [c] 26.58	20.46	[d] 28.44, [e] 28.60
(c) Extract, 12.25 per cent sodium chlorid	[b] 54.76, [c] 53.46	50.43	[d] 54.68, [e] 54.50

[a] Without sand, seventy-three hours in vacuum desiccator.
[b] Three hours in oven as for (a) in preceding table, using sand.
[c] After seventy-three hours in vacuum desiccator, dried two hours in vacuum oven as for (a) in preceding table, without sand.
[d] Thirty minutes on steam bath and one hour in air oven at 105° C.
[e] Thirty minutes on steam bath and one hour in oven as under (a) in preceding table.

With the official methods for the determination of moisture good duplicates are readily obtained when the same degree of heat is used for the same length of time and the weight of the sample does not vary too much. In many cases, however, the weight considered as the dry weight is not the final constant weight and therefore represents only an empirical determination.

In many food-stuff analyses other determinations are to be made on the sample freed from moisture, and the heat used to remove the moisture frequently drives off other substances or changes the nature of the remaining substance in such a manner as to interfere with subsequent determinations. When the moisture is removed by the vacuum method without heat fats are

not oxidized, soluble proteins are not coagulated, organic phosphorus compounds are not changed to inorganic forms, etc. The length of time required to complete the determination by the vacuum method is a serious objection if comparative control only is wanted; but when accurate determinations are wanted, as in all investigations, the method should be employed when any substances may be present which can in any way be affected by the heat used in the official method.

Recommendations.

It is recommended that—

(1) The referee on this subject for next year test the vacuum method (without heat) in connection with the official method to determine what class of substances give incorrect data by the latter; also that the applicability of the Lowenstein alcohol method to certain classes of food products be studied.

(2) The methods for moisture determination be further studied with a view to determining their effect on the determination of the fat in food products.

REPORT ON THE SEPARATION OF MEAT PROTEIDS.

By P. F. Trowbridge, *Associate Referee.*

The report of the referee on the separation of meat proteids is a continuation of the work outlined a year ago.[a] Samples of beef extract of known origin were sent to nine chemists who had signified a desire to cooperate. The following directions were sent at the same time as a guide for the work:

Directions for Analysis of Samples of Beef Extract.

Moisture.—Tare a crucible one-half full of ignited sand with a short stirring rod. Weigh out 2 or 3 grams of the sample by difference, mix thoroughly with the sand, and dry to constant weight at not to exceed 105° C. in an atmosphere of hydrogen or in vacuo; or dry in vacuo in desiccator over sulphuric acid at room temperature. If time allows compare with the alcohol method.[b]

Ash.—Ignite about a 2-gram sample in a crucible to complete combustion of all organic matter. Use as low a heat as possible to avoid volatilization of chlorids. It will help to bake the samples before igniting.

Total phosphorus.—Determine the total phosphorus in the ash samples as in the gravimetric method for total phosphoric acid in fertilizers. Report as the element phosphorus.

Organic phosphorus.—Dissolve about 10 grams in a 300 cc beaker (do not heat), transfer to a 500 cc flask. Have a volume of about 450 cc, add ammonium hydroxid (1:1) to slight alkalinity, and barium chlorid solution till no further precipitate is formed. Fill to the mark, mix thoroughly, and allow to stand two hours or overnight. Filter through a dry filter, returning the first portion of the filtrate to the filter. Transfer 450 cc of the filtrate to 800 cc nitrogen flasks, add 25 cc of concentrated sulphuric acid and 0.7 gram of mercury and digest as for Kjeldahl determinations. Transfer the residue to 400 cc beakers with water, neutralize with ammonium hydroxid, and determine the phosphorus gravimetrically as for fertilizers. Multiply by 10/9 to obtain the phosphorus in the entire sample.

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[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. 22, p. 61.

[b] Lowenstein, J. Ind. Eng. Chem., 1909, 1:252.

Inorganic phosphorus is determined by difference.

Total nitrogen.—Weigh out about 2 grams of the sample and digest in a 500 cc nitrogen flask with 25 cc of sulphuric acid, 0.7 gram of mercury, and about 7 grams of potassium sulphate, adding the latter after frothing has ceased. When the digestion is apparently complete, cool, rinse down the flask, and boil again for one hour. Transfer the cooled residue to a 250 cc flask using ammonia-free water, make up to the mark, mix thoroughly, and determine the ammonia in 50 cc portions in the usual manner. Make a blank determination with all reagents.

Soluble nitrogen.—Dissolve about 10 grams of the extract in water, do not heat, make up to 500 cc, mix thoroughly, and filter through a dry filter returning the first portion of the filtrate. This is filtrate (A). Determine the nitrogen in 25 cc portions.

Coagulable nitrogen.—To 100 cc of the filtrate (A) in 200 cc beakers, add a slight excess of moist precipitated magnesium carbonate and concentrate on the water bath to about 20 cc. Filter and wash any coagulum present with hot water saturated with magnesium carbonate. Determine the nitrogen in the coagulum.

Amido acid nitrogen.—Concentrate the filtrate and washings from the coagulum above to about 20 cc. Transfer to a 100 cc flask. Volume should not exceed 50 cc, add 15 grams of sodium chlorid and shake thoroughly, wash down the neck of the flask and cool for several hours in the ice box. Prepare and filter a 24 per cent solution of tannic acid, cool in the ice box. Also cool a flask of distilled water. Add 30 cc of the cold tannic-acid solution to each sample, fill to the mark with cold distilled water, mix thoroughly and allow to stand overnight in the ice box. Filter rapidly through a dry filter and immediately draw 50 cc with a pipette for the determination of the nitrogen not precipitated by the tannic acid. Transfer the 50 cc to 800 cc nitrogen flasks, add 35 cc of sulphuric acid and 0.7 gram of mercury. Heat gently until the violent frothing is past, then continue the digestion. It is not necessary to add potassium sulphate. Make blank determinations with the reagents. Calculate the results to per cent amido nitrogen in the original substance.

Acidity.—Take 10 cc of the filtrate (A), dilute with recently boiled distilled water to which sufficient twentieth-normal sodium hydroxid has been added to give a faint end reaction with phenolphthalein. Titrate with tenth-normal sodium hydroxid and report number of cubic centimeters required to neutralize 100 grams of sample.

Creatinin.[a]—Transfer 20 cc of solution (A) to 500 cc measuring flasks, add 25 cc of 1.2 per cent picric acid solution, mix, add 10 cc of a 10 per cent solution of sodium hydroxid, shake one-half minute and allow to stand exactly four and one-half minutes, dilute to the mark, immediately mix, and compare the color of the solution with that of a half-normal potassium bichromate solution set at 8 mm.

Creatin (combined creatinin and creatin).—Transfer 50 cc of solution (A) to a 100 cc flask, add 25 cc of normal hydrochloric acid, and heat for twenty-five minutes in an autoclave at 117° to 119° C. Allow to cool completely and add 25 cc of normal sodium hydroxid (make the volume exactly 100 cc). Mix thoroughly and with an aliquot portion proceed as for creatinin. It will probably be necessary to use about 40 cc in order that the reading may be about 8 mm on the colorimeter. Apply Cook's correction if a large aliquot is used, or concentrate and use a smaller aliquot. Subtract from the combined creatinin value the equivalent of the preformed creatinin and multiply the difference by 1.16 to convert into creatin. Report the results as follows, in per cents:

Moisture, state method used; total ash; total phosphorus; organic phosphorus; inorganic phosphorus; total nitrogen; soluble nitrogen; coagulable nitrogen (soluble); amido acid nitrogen; creatinin and creatin. Report acidity in cubic centimeters of tenth-normal sodium hydroxid necessary to neutralize a 100-gram sample.

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[a] Emmett and Grindley, J. Biol. Chem., 1907, **3**: 514, and F. C. Cook, J. Amer. Chem. Soc., 1909, **31**: 692.

Discussion of Results.

Some valuable suggestions and criticisms have been reported by different workers, the most important of which are quoted:

MOISTURE.

A. Lowenstein states:

Owing to the crystals of potassium phosphate which had separated out it was difficult to get samples sufficiently uniform to obtain accurate moisture determinations. * * * This was the first sample of meat extract that we have encountered which we could not dry to constant weight in one hour and thirty minutes by the alcohol method. We seem to get the same results by the alcohol method by drying in an air oven at 105° C. or in a vacuum oven at 100° C. * * * Drying in a vacuum desiccator over c. p. sulphuric acid, with frequent agitation, gave low results on two samples dried without admixture. The addition of 95 per cent alcohol did not seem to help any.

Mr. Lowenstein's results on moisture are particularly interesting and are discussed in full in the report of the referee on moisture in foods (page 150). He apparently obtained a uniform sample, since with eight moisture determinations by modified methods but all using heat he gets an average of 26.44 per cent of moisture, with a maximum of 26.67 per cent and a minimum of 26.12 per cent. However, by the vacuum method over sulphuric acid without heat he reports 22.4 per cent and 22.3 per cent. In our laboratory, on the same sample, C. R. Moulton finds 21.48 and 21.34 per cent.

W. D. Richardson was not able to cooperate in the work, but submitted a criticism of the methods proposed, the more important points of which will be noted under the different determinations. He thinks that not less than 5 or 10 grams should be taken for moisture determinations, and that they should be made in a shallow dish. If the moisture is determined by aid of heat a larger sample may be used. By the vacuum method without heat 2 or 3 grams is better. With a larger sample there is unnecessary dilution of the sulphuric acid. The agreement of duplicates is evidence that the sample is large enough. Thorough mixing to secure uniformity of sample is most important, and all samples should be obtained by weighing by difference. If sand is not used a shallow dish is essential.

ASH.

Mr. Richardson condemns the proposed method as certain to yield incorrect results owing to the impossibility of burning off the carbon. He is correct, and before his criticism was received the method had been modified as follows:

Char the sample completely at a low heat, cool, extract with hot water, and filter through an ashless filter; wash out all the soluble ash with hot water. Burn the contents of the filter to a complete ash and weigh. Evaporate the filtrate on the steam bath in a tared platinum dish, ignite to a dull red heat, and weigh. Combine the weights for total ash.

On one sample 20.02 and 19.86 per cent of ash was found, and on the same sample Mr. Lowenstein reports 19.55 per cent for one chemist and 20.95 for another. On another extract 19.68 and 19.50 per cent were found at the Missouri station as compared with 19.79 per cent reported by Byron McClelland of the New York Food and Drug Inspection Laboratory.

PHOSPHORUS.

Mr. Richardson suggests a larger sample for total phosphorus than the ash from the 2-gram sample. Our experience would point to the use of a smaller rather than a larger sample. In one case we find 2.99 and 3.06 per cent of

total phosphorus as compared to 3.05 and 3.03 reported by Mr. Lowenstein's two chemists. In another sample we find 2.85 and 2.90 per cent as compared to 1.73 per cent reported by Mr. McClelland.

Lowenstein reports 0.43 per cent of organic phosphorus, which is exactly the average of our duplicates, which, however, do not agree closely, i. e., 0.361 and 0.505. Mr. McClelland finds in his sample 1.66 per cent of organic phosphorus, while we find 0.445 per cent, an unexplainable discrepancy.

NITROGEN.

Mr. Richardson thinks that the determination should be continued with the 2-gram sample instead of taking a fifth aliquot. The referee does not agree with him, as the sample contains from 7 to 9 per cent of nitrogen and the fifth aliquot requires 20 to 30 cc of tenth-normal acid, which is certainly sufficient for the most accurate nitrogen determinations. Furthermore, it is difficult to obtain complete dissociation of the amido nitrogen bodies to ammonia, and long continued digestion with both the mercury and the potassium sulphate is suggested. In one sample we find 8.49 and 8.60 per cent, as compared with 8.63 and 8.75 per cent reported by Lowenstein's chemists. McClelland reported 8.30 per cent, while at the Missouri station 7.90 and 7.92 per cent were found.

AMIDO ACID NITROGEN.

Mr. Richardson comments as follows:

This method, after many trials by good workers, has been discarded by both Professor Grindley and myself. Not only does it yield inconsistent results, but the fundamental fact that the tannin contains too much nitrogen for use when determining nitrogenous constituents makes the method unworthy of consideration unless special directions are given for purifying the tannin. We feel very sure that good results are not possible by this method. It is certain that the temperature of precipitation exerts some influence, and the instructions are not specific on this point; also the tannin used exerts some influence.

We agree that the method leaves much to be desired, but think that the results have some value in determining the approximate limits of this form of nitrogen in comparison with the total nitrogen present. Our duplicates are quite close, although they do not agree particularly well with those of Lowenstein or McClelland. Lowenstein reports 4.88 per cent; we find 3.96 and 3.98. McClelland reports 3.94 per cent; we find 4.28 and 4.24.

CREATININ.

Mr. Richardson says:

We have done considerable work on this method and have read the various papers which have been written on it. The amount of work which has been done on this method seems out of proportion to its value and usefulness, and the cooperative results obtained thus far do not warrant further trials unless further facts in regard to the method are developed. * * * The work of Chapman, reported on page 492 of the July number of the Journal of Industrial and Engineering Chemistry, shows that the method, depending as it does on a variable reduction reaction, is scarcely to be relied upon.

From a commercial point of view it is quite possible that at the present time the determinations of creatinin and creatin are not of much value. However, it can hardly be disputed that the attempts to perfect the method for the estimation of these bodies should be continued. At the Missouri station we have tried to profit by the experience and suggestions of Cook and of Emmett and Grindley. We find no difficulty in getting a good agreement of triplicates but

find that preliminary experimentation is necessary to ensure the use of sufficient material so that the reading on the Duboscq colorimeter is approximately 8 mm, otherwise the results are inaccurate. Our results do not agree with those reported by Lowenstein or McClelland, as the following table shows:

Cooperative results on creatinin and creatin.

Analyst.	Creatinin.	Creatin.
	Per cent.	*Per cent.*
McClelland (sample 152)	6.75	3.92
Moulton (sample 152)	2.37	2.70
Lowenstein (sample 160):		
W. P. Dunne, chemist	2.21	2.62
A. L. Nehls, chemist	2.14	2.92
Moulton (sample 160)	2.16	4.00

McClelland reports the reading for creatinin as 3 mm and the reading after conversion as 2 mm on the colorimeter. These readings are so far from the 8 mm used as a standard that good results can not be expected. Lowenstein does not report his readings. In order to compare results it is essential that the aliquot be taken so as to give a reading of approximately 8 mm. The disagreement is not encouraging, but if one chemist can get good results on duplicates others ought to be able to get concordant results if care is taken and if the directions are given in sufficient detail.

In Mr. Moulton's work at the Missouri station the details on one sample (No. 160) will show the agreement of the duplicates:

For creatinin 20 cc duplicates of solution A, representing 0.4375 gram of the extract, were used. (a) Of 8 readings the highest was 8.9 mm, the lowest 8.3 mm; (b) of 8 readings the highest was 8.9 mm, the lowest 8.3 mm; average of 16 readings was 8.575 mm or 2.16 per cent.

For creatin 50 cc duplicates of solution A were heated in the autoclave, and after cooling were made up to 100 cc. Of this solution 20 cc were used for the readings in the colorimeter with the following results: (a) of 6 readings the highest was 6.9 mm, the lowest 6.4 mm; (b) of 6 readings the highest was 6.8 mm, the lowest 6.5 mm; average of 12 readings, 6.6 mm, equivalent to 12.273 mg of creatinin in 10 cc of solution A. Subtracting for the original creatinin and converting to creatin the result is 4 per cent.

At a later date the referee sent to F. C. Cook, of the Bureau of Chemistry, four samples of beef extract of known origin on which he made the usual determinations for such material. The results are incorporated here for comparison with those obtained by Mr. Moulton in this laboratory.

Cooperative results in four samples of beef extracts.

Determinations.	Sample No. 153.		Sample No. 158.		Sample No. 161.		Sample No. 162.	
	Moulton.	Cook.	Moulton.	Cook.	Moulton.	Cook.	Moulton.	Cook.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Total nitrogen	8.74	9.43	8.40	9.43	8.88	8.63	9.38	9.13
Amido-acid nitrogen	4.24	6.39	4.12	6.03	4.09	6.74	4.16	6.74
Creatin	2.60	2.90	3.68	4.00	1.62	1.51	1.13	1.95
Creatinin	4.26	2.60	3.02	2.22	2.89	2.35	4.20	2.62
Ash	20.82	16.23	19.87	19.57	18.13	14.63	21.36	14.49
Phosphorus, total	2.81	2.63	2.86	2.30	2.27	1.96	2.97	2.22
Phosphorus, organic	.37	.29	.49	.24	.20	.25	.22	.27

In all samples containing a large amount of amido nitrogen there is difficulty in getting accurate results. Both mercury and potassium sulphate should be used and the digestion should be continued at least three hours. An effort should be made to perfect the method for creatin and creatinin so that it may receive official sanction, and it is recommended that the referee for next year make a special study of methods that should be used in the examination of meat extracts and similar preparations.

PHOSPHORUS IN FLESH.[a]

By P. F. Trowbridge and Louise M. Stanley.

Hart and Andrews[b] were among the first to show that the phosphorus in foods is present to a considerable extent in the organic form. They precipitated the inorganic phosphorus with neutral ammonium molybdate in the presence of ammonium nitrate and a small excess of nitric acid, and concluded that in vegetable feeding stuffs nearly all of the phosphorus is in the organic form.

Experiment shows that, with a known amount of inorganic phosphorus only, neutral molybdate solution fails to precipitate the whole of the phosphorus in the presence of 10 grams of ammonium nitrate and 2 cc of concentrated nitric acid.

Emmett and Grindley[c] modified the method for application to the analysis of cold water extracts of flesh. The soluble proteins made it difficult to use the method direct. These were removed by evaporating the sample of the cold water extract to a small volume, nearly neutralizing the solution, and filtering off the coagulum which was thoroughly washed with neutral water. The combined filtrate and washings, concentrated if necessary, were examined for organic phosphorus by the use of the neutral molybdate solution.

They found a satisfactory agreement of duplicates even when a considerable amount of nitric acid (20 cc) was used. However, their results indicated that only a small amount of the soluble phosphorus in flesh is in the organic form.

In this laboratory the method of Emmett and Grindley was followed for more than a year with results which seemed to indicate that there was practically no organic phosphorus present in that portion of beef soluble in cold water.

In the fall of 1908 a study was made of the method proposed by Siegfried and Siegewald,[d] for the separation of inorganic from organic phosphorus by the precipitation of the former with barium chlorid and ammonium hydroxid and the estimation of the phosphorus in an aliquot of the filtrate as organic phosphorus.

In the preliminary experiment with a cold water extract of a piece of beef chuck 85.5 per cent of the total soluble phosphorus was found to be organic by Siegfried's method and only 7.2 per cent organic by Emmett and Grindley's method. In another sample 86.3 per cent of organic phosphorus was found by Siegfried's method, as compared with 0.86 per cent by Emmett and Grindley's method; and in still another sample we find 57.4 per cent by the former method, as compared with 6.3 per cent by the latter method.

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[a] See J. Ind. Eng. Chem., 1910, **2**: 212, for detailed report on this work.

[b] Amer. Chem. J., 1903, **30**: 470.

[c] J. Amer. Chem. Soc., 1906, **28**: 25.

[d] Zts. Nahr. Genussm., 1905, **10**: 521.

Portions of the last two samples of meat were made into beef loaves and cooked. The loaves were ground and mixed and cold water extracts prepared. These showed respectively 17.6 and 16.5 per cents of organic phosphorus by the Siegfried method, as against 7.4 per cent and a trace by Emmett and Grindley's method.

The determinations of the organic phosphorus in water extracts of flesh by the Siegfried method indicates the presence of a very considerable quantity, which amount is very much less in extracts prepared from cooked meats. This would seem to indicate that the heat causes a decomposition of the organic phosphorus compound, changing it into a form precipitable as inorganic phosphorus. Since the Emmett and Grindley method calls for the concentration of the extract upon the water bath, the action of the heat would explain the small amounts of organic phosphorus as shown by them.

These results seemed to be of sufficient importance to warrant a more extensive experiment to determine the nature of the phosphorus in fresh meats. Numerous determinations were made upon water extracts prepared from fresh meats, roasts, and beef loaves cooked at different temperatures. The results warrant the following conclusions:

(1) Fresh lean beef contains about 0.2 per cent of phosphorus (usually a little less).

(2) About 75 per cent of the total phosphorus in lean flesh, raw or cooked, is soluble in cold water.

(3) About 75 per cent of the phosphorus in the cold water extracts of the raw meats is in the organic form.

(4) When meat is cooked in the form of roasts or beef loaves the heat of cooking changes the greater portion of the organic phosphorus to the inorganic form. The higher the temperature the meat has reached, the greater the decomposition of the organic phosphorus.

If the interior of the roast or beef loaf has reached 60° to 65° C., only about 25 per cent of the soluble phosphorus is still in the organic form. At 98.5° C. one experiment showed only 9.82 per cent of the soluble phosphorus still in the organic form.

Mr. Trowbridge and Mr. Waters also presented a preliminary report on four series of experiments conducted at the Missouri station on Factors Influencing the Digestibility of Feed, with special reference to the effect of the quantity of feed. Details of these experiments are to be presented as a Missouri experiment station bulletin. The results of the twenty-two digestion trials so far completed are, in brief, as follows:

Series 1—Influence of quantity of feed.—Excessive quantity of food reduces the digestion coefficient even when the undigested grain washed from the dung is deducted as if never consumed. Animals in a healthy but extremely emaciated condition also have a low digestion coefficient.

Series 2—Influence of age.—Young steers on full feed apparently digest better than old ones also on full feed. Of steers in good medium thrifty condition the mature steers show a decided gain over the younger ones.

Series 3—Influence of condition.—Animals excessively fat or excessively thin have a low capacity to digest feed compared with animals in a medium condition.

Series 4.—Mature dairy cows producing milk and consuming all they can eat without gaining in body weight (practically full fed) have a low digestion coefficient—about the same as that of very fat full-fed steers. The same cows

when dry and not pregnant and on the same food reduced in quantity so as to maintain the same body weight show a high digestion coefficient.

Animals seem to vary as to their ability to digest the different food constituents. The steer having the highest total digestion coefficient (83.75 per cent) is first in carbohydrates (91.059 per cent), second in protein (88.502 per cent), and crude fiber (49.193 per cent), and seventh in fat (82.032 per cent). He was a steer about eighteen months old, in good condition, and on maintenance at 1,100 pounds. The steer showing the lowest total digestion coefficient (67.163 per cent) is lowest in carbohydrates (71.598 per cent) and protein (61.639 per cent), fourth from lowest in fat (75.868 per cent), and sixth from highest in crude fiber (43.320 per cent). He was the oldest animal in the trials and on full feed, weighed 1,250 pounds at time of the trial.

CONSTANTS FOR KREATININ DETERMINATIONS.

By W. B. Smith and I. M. Myers.

While the methods for the determination of kreatinin have been extensively studied in the last few years, there has been no attempt to substitute other colorimeters for the Duboscq. One reason for this is the difficulty of obtaining pure kreatinin with which to standardize the solution of bichromate. The objects of this paper are to show that any other colorimeter may be used as well as the Duboscq, and to give kreatinin values for standard bichromate solutions.

The solutions used by Folin were a 0.5 normal solution of potassium bichromate and a solution of kreatinin containing 10 mg in 500 cc. When compared in the Duboscq colorimeter 8 mm of bichromate are equal to 8.1 mm of kreatinin. The Duboscq comparison tubes are, however, very much less in depth and width than tubes of the Nessler type, and it is to be expected that the ratio of kreatinin to bichromate found by Folin will not hold true with columns of solutions of so much greater dimensions.

In order to obtain exact data on this point, solutions of bichromate and kreatinin were compared in an ordinary colorimeter having comparison tubes 28.5 mm inside diameter and graduated in an arbitrary scale each division of which is 1.5 mm in depth. To avoid confusion, however, all readings given in this paper are expressed in millimeters. No change was made in the concentration of the bichromate, as 0.5 normal was found to give the best results in enabling the eye to judge of color changes. The kreatinin solutions were prepared from crystallized kreatin, the percentage of kreatinin being determined by the aid of a Duboscq colorimeter.[a] Dehydration of the kreatin was effected either by heating 10 cc with 10 cc of normal hydrochloric acid for four hours on a water bath or by evaporating 25 cc with 12.5 cc of normal hydrochloric acid twice. Other details of the determination were carried out exactly as recommended by the best authorities.[b]

The kreatinin solutions, after having been made up to 500 cc, were compared with the bichromate at depths of from 30 mm to 150 mm at intervals of 15 mm. From three to ten readings were made at each depth with each solution, several hundred comparisons being made altogether. By dividing the reading of the kreatinin by that of the bichromate and multiplying the result by the weight of kreatinin in milligrams per 500 cc, the strength of kreatinin solution required to read the same as the bichromate was obtained.

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[a] The kreatin was found to be 95.36 per cent pure.

[b] Cook, J. Amer. Chem. Soc., 1909, **31**: 673.

TABLE 1.—*Kreatinin values of bichromate.*

Readings (mm).		Mg per 500 cc.		Readings (mm).		Mg per 500 cc.	
Bichromate.	Kreatinin.	Strength of kreatinin.	Kreatinin value of bichromate.	Bichromate.	Kreatinin.	Strength of kreatinin.	Kreatinin value of bichromate.
30	19.5	9.536	6.20	90	89.0	4.768	4.71
30	31.5	5.722	6.01	90	45.0	9.536	4.77
30	37.5	4.768	5.96	90	72.0	5.722	4.58
30	49.5	3.814	6.29	90	88.5	4.768	4.69
30	50.7	3.61	6.11	90	102.0	4.10	4.65
30	44.5	4.10	6.08	90	114.9	3.61	4.61
30	42.0	4.32	6.05	90	96.3	4.32	4.62
Average			6.12	Average			4.66
45	27.0	9.536	5.72	105	100.5	4.768	4.56
45	127.5	1.907	5.40	105	51.0	9.536	4.63
45	42.0	5.722	5.34	105	82.5	5.722	4.49
45	52.5	4.768	5.56	105	99.0	4.768	4.49
45	64.5	3.814	5.47	105	123.0	3.814	4.47
45	60.0	4.10	5.46	105	128.4	3.61	4.42
45	69.0	3.61	5.53				
45	56.1	4.32	5.39	Average			4.51
45	36.0	7.21	5.77				
45	52.5	4.768	5.56	120	111.0	4.768	4.41
45	89.7	2.86	5.70	120	91.5	5.722	4.36
				120	109.5	4.768	4.35
Average			5.54	120	145.5	3.61	4.38
				120	120.0	4.32	4.32
60	54.0	5.722	5.15	120	142.0	3.814	4.51
60	70.9	4.32	5.11				
60	66.0	4.768	5.24	Average			4.39
60	123.0	2.46	5.04				
60	73.5	4.10	5.03	135	100.5	5.722	4.26
60	85.8	3.61	5.16	135	123.0	4.768	4.34
60	43.2	7.21	5.19	135	134.3	4.32	4.30
60	65.0	4.768	5.17				
				Average			4.30
Average			5.14				
75	39.0	9.536	4.96	150	132.0	4.768	4.20
75	63.0	5.722	4.81	150	67.5	9.536	4.29
75	76.5	4.768	4.86	150	111.0	5.722	4.23
75	96.0	3.814	4.88	150	133.5	4.768	4.24
75	88.5	4.10	4.84				
75	98.1	3.61	4.72	Average			4.24
75	82.0	4.32	4.72				
75	136.2	2.67	4.85				
75	77.0	4.768	4.89				
Average			4.84				

TABLE 2.—*Kreatinin equivalents (calculated from Table 1 and from curve.)*

Readings.	Kreatinin.	Readings.	Kreatinin.	Readings.	Kreatinin.
mm.	*mg per 500 cc.*	*mm.*	*mg per 500 cc.*	*mm.*	*mg per 500 cc.*
30	6.12	75	4.84	120	4.39
35	5.92	80	4.77	125	4.36
40	5.72	85	4.71	130	4.33
45	5.54	90	4.66	135	4.30
50	5.38	95	4.61	140	4.28
55	5.26	100	4.56	145	4.26
60	5.14	105	4.51	150	4.24
65	5.02	110	4.47		
70	4.92	115	4.43		

The results take the form of a regular curve (fig. 7), showing that the intensity of color of the kreatinin increases at a more rapid rate than that of the bichromate, this increase in rate being much less at 150 mm than at 30 mm. It is shown that a solution of kreatinin containing 6.12 mg in 500 cc will read 30 mm against 30 mm of bichromate, while the required amount of kreatinin decreases with increasing depth until at 150 mm only 4.24 mg are required. It will be noticed that this concentration is about one-half that of Folin's solution.

Having obtained the equivalent in kreatinin of a given depth of bichromate, it remains to apply these figures to unknown solutions. After making the readings at one or several depths the reading of the bichromate is divided by that of the kreatinin, and this figure is multiplied by the kreatinin equivalent

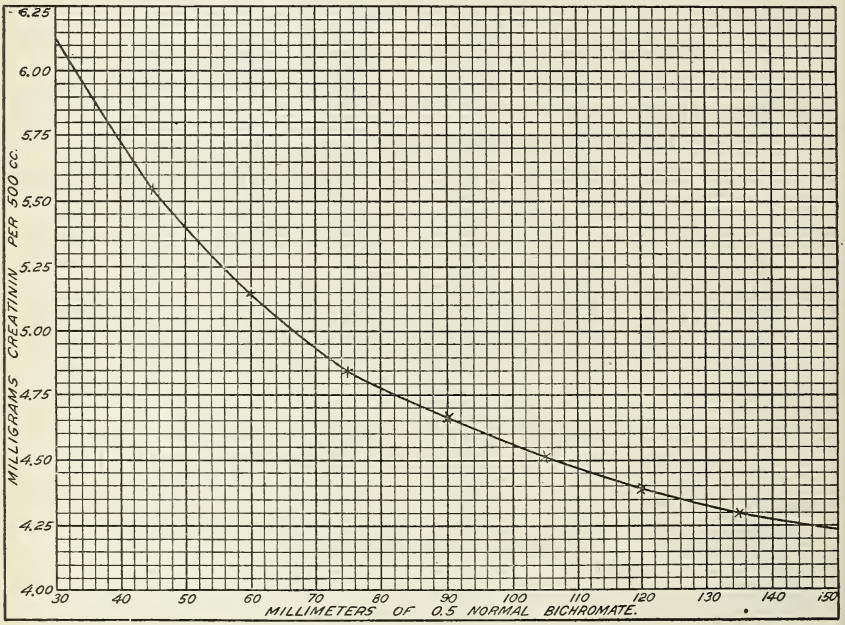

FIG. 7.—Kreatinin equivalent of 0.5 normal bichromate.

of the bichromate reading, the result being the number of milligrams of kreatinin in the whole 500 cc of the final solution.

It is immaterial at what depth the readings are made, the elasticity of the method in this respect increasing its serviceability considerably. Moreover, the wide range in depth is an aid to accuracy, as if at one depth the turbidity of the sample solution, or some other cause, prevents matching the shades with exactness, it is possible, by reading at other depths, to decide which figures are the most nearly correct. The best results are obtained with solutions containing approximately 5 mg of kreatinin. Of three known solutions, each of which was read at six or eight different depths, the maximum error was +0.11 mg and the minimum ±0.00.

TABLE 3.—*Experimental results on three solutions.*

Readings.		Kreatinin equivalent of bichromate.	Kreatinin found.	Kreatinin taken.	Error.
Bichromate.	Kreatinin.				
mm.	*mm.*	*mg per 500 cc.*	*mg per 500 cc.*	*mg per 500 cc.*	
Solution 1:					
30	31.5	6.12	5.83	5.72	+0.11
60	54.0	5.14	5.71	5.72	− .01
75	63.0	4.84	5.76	5.72	+ .04
90	72.0	4.66	5.82	5.72	+ .10
105	82.5	4.51	5.74	5.72	+ .02
120	91.5	4.39	5.76	5.72	+ .04
135	100.5	4.30	5.78	5.72	+ .06
150	111.0	4.24	5.73	5.72	+ .01
Average			5.77	5.72	+ .05
Solution 2:					
45	52.5	5.54	4.75	4.77	− .02
60	66.0	5.14	4.67	4.77	− .10
75	76.5	4.84	4.75	4.77	− .02
90	88.5	4.66	4.74	4.77	− .03
105	99.0	4.51	4.78	4.77	+ .01
120	109.5	4.39	4.81	4.77	+ .04
Average			4.75	4.77	− .02
Solution 3:					
30	50.7	6.12	3.65	3.61	+ .04
45	69.0	5.54	3.61	3.61	.00
60	85.8	5.14	3.59	3.61	− .02
75	98.1	4.84	3.70	3.61	+ .09
90	114.9	4.66	3.65	3.61	+ .04
105	128.4	4.51	3.69	3.61	+ .08
120	145.5	4.39	3.62	3.61	+ .01
Average			3.64	3.61	+ .03

It is, however, important that the solutions should be of nearly the same depth, as in this method of calculation it is assumed that the kreatinin-equivalent curve is a straight line, which is, of course, not so. Ordinarily the error is within the limits of the ability of the eye to distinguish color differences, but if the kreatinin reading is as much as twice that of the bichromate the results are considerably too high, while if the kreatinin is so strong as to give a very low reading compared to the bichromate the results are too low.

TABLE 4.—*Effect of weak and strong samples on kreatinin results.*

(Milligrams per 500 cc.)

(a) Weak.					
Readings.		Kreatinin equivalent.	Kreatinin found.	Kreatinin taken.	Error.
Bichromate.	Kreatinin.				
mm.	*mm.*				
30	90	6.12	2.08	1.91	+0.17
45	120	5.54	2.15	1.91	+ .24
30	69	6.12	2.66	2.46	+ .20
(b) Strong.					
60	34.5	5.14	8.94	9.54	−0.60
120	57	4.39	9.25	9.54	− .29
135	63	4.30	9.23	9.54	− .31

This set of constants has been used satisfactorily with meats and meat extracts for two years. In order to check the figures, a sample of high-grade commercial beef extract was divided into two portions, one of which was sent to the Washington laboratory of this division,[a] while the other was analyzed in South Omaha. In Washington the kreatin and kreatinin were determined in a Duboscq colorimeter, the kreatin being converted into kreatinin by the autoclave method. In South Omaha the modified method was employed, 120 mg of the extract being used for determining preformed kreatinin. For total kreatinin 80 mg, having a volume of 25 cc, were evaporated with 12.5 cc normal hydrochloric acid twice. The figures given in Table 5 were obtained.

TABLE 5.—*Kreatinin results on commercial beef extract.*

Readings.		Kreatinin.			Weight of sample.
Bichromate.	Kreatinin.	Equivalent.	Amount found.	Per cent found.	
mm.	*mm.*	*mg per 500 cc.*	*mg per 500 cc.*		*mg.*
Preformed:					
30	35.7	6.12	5.15	4.29	120
45	49.5	5.54	5.04	4.20	120
60	61.5	5.14	5.01	4.18	120
75	72.7	4.84	4.99	4.18	120
Average				4.21	
Total:					
Portion 1–60	53.4	5.14	5.78	7.23	80
Portion 1–45	43.1	5.54	5.79	7.24	80
Portion 2–60	54.0	5.14	5.71	7.14	80
Portion 2–45	43.1	5.54	5.79	7.24	80
Portion 2–75	63.0	4.84	5.76	7.20	80
Average				7.21	

SUMMARY.

Laboratory.	Preformed kreatinin.	Total kreatinin.	Kreatinin from kreatin.	Kreatin.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
South Omaha, Nebr	4.21	7.21	3.00	3.48
Washington, D. C	4.27	7.26	2.99	3.46

The color comparison tubes used in this work were those of the Kennicott-Sargent colorimeter, their inside diameter being 28.5 mm. The tubes of the Schreiner instrument are on the average about 25 mm in diameter, and this difference in the width of the column of solution makes the results, when this colorimeter is used, too low by approximately 1 per cent of the kreatinin found. A correction can easily be applied, however, for this error, which, even in the case of beef extracts containing over 7 per cent of total kreatinin, is within one-tenth of 1 per cent.

REPORT OF FINANCE COMMITTEE.

It is recommended—

(1) That each agricultural college, experiment station, and each other body whose chemist is entitled to vote in this association be requested to pay to the

[a] Biochemic Division, Bureau of Animal Industry, U. S. Department of Agriculture.

secretary, the sum of $2 for the purpose of meeting the expenses already incurred in printing and sending out notices of this meeting and other incidental expenses, up to and including the next meeting, which were met in previous years by the U. S. Department of Agriculture, but which under recent rulings can no longer be met by the department, unless Congress makes an appropriation for this purpose.

(2) That a committee of three be appointed by the president of the association to ask the approval of the Secretary of Agriculture of a request to Congress to appropriate the sum of $500 annually to the U. S. Department of Agriculture for the purpose of meeting such expenses of this association as can not now be lawfully met by the department.

E. M. Magruder.
J. P. Street.
H. A. Huston.

This report of the finance committee was unanimously adopted by the association and the following committee to carry out its provisions has been appointed by President Withers: J. P. Street, H. E. Barnard, and R. J. Davidson.

REPORT OF COMMITTEE C ON RECOMMENDATIONS OF REFEREES.

By A. L. Winton,[a] *Chairman.*

Water in Foods.

It is recommended—

(1) That the referee on this subject for next year test the vacuum method (without heat) in connection with the official method to determine what class of substances give incorrect data by the present official method; that the applicability of the Lowenstein alcohol method to certain classes of food products be also studied.

Adopted.

(2) That the methods for moisture determination be further studied with a view to determining their effect on the determination of the fat in food products.

Adopted.

Separation of Nitrogenous Bodies—Meat Proteids.

It is recommended—

(1) That the referee for next year on the separation of meat proteids pay special attention to the development of methods. Details of manipulation should be carefully worked out, especially with regard to determinations of amido acids, kreatinin, and kreatin.

Adopted.

(2) That an associate referee be appointed to make a special study of the inorganic and organic phosphorus compounds in foods, paying especial attention to the development of methods.

Adopted.

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[a] In the absence of Mr. Winton the report was presented by J. P. Street, Connecticut.

Flavoring Extracts.

It is recommended—

(1) That the provisional method for the determination of citral in lemon extracts be adopted as an official method.

Approved and referred to the asssociation for final action in 1910.

Meat and Fish.

It is recommended—

(1) That the referee give special attention to the apparent error in the method for the determination of sugar in meat and meat products noted by Lowenstein in the Journal of the American Chemical Society, September, 1908.

Adopted.

(See also the report of the referee on meat and fish (page 119) for recommendation in regard to work not received in time to be acted upon by the committee.)

Dairy Products.

It is recommended—

(1) That the modified Baier and Neumann method for sugar in cream and milk be brought before the association next year and considered for provisional adoption.

Adopted.

(2) That a further study be made of the methods for determining calcium oxid and the alkalinity of ash in cream.

Adopted.

(3) That a study be made of the copper method of preparing milk serum.

Adopted.

REPORT OF COMMITTEE ON THE UNIFICATION OF THE METHODS OF ANALYSIS OF FATS AND OILS.

By L. M. Tolman, *Chairman.*

This committee, consisting of the chairman and Messrs. P. H. Walker, of Washington, D. C., and A. Lowenstein, of Chicago, Ill., was appointed at the last meeting of the Association of Official Agricultural Chemists with the object of bringing about a cooperation between this association and the American Chemical Society and the American Society for Testing Materials, in order to prevent needless duplication of work and to agree upon uniform methods of analysis and reporting results; also to insure participation in the organization of an international commission for the same objects. Your committee has united with similar committees of three appointed by the American Chemical Society and by the American Society for Testing Materials, thereby forming what is known as the Joint Committee on the Unification of Methods of Analysis of Fats and Oils. This joint committee has taken up the question of the formation of an international commission and at present is cooperating to this end with similar organizations in most of the countries of Europe. The formation of such an organization will undoubtedly be accomplished this coming year.

The necessity of the unification of the methods of analysis to be used in this country is apparent to all, but it is even more necessary that some agreement be reached as to the methods to be used in judging fats and oils exported to

foreign countries. We ship great quantities of edible fats and oils to foreign countries, and on the basis of a chemical analysis there is a constant disagreement between the buyer and seller, largely caused by the difference in methods of analysis employed in different countries. It is the object and aim of this international commission to bring about some agreement as to the methods of analysis employed.

The plan of the work of the joint committee is to take up various methods, such as the determination of specific gravity, and by extended correspondence with chemists interested in the subject obtain an exact knowledge of the condition of affairs in this country, with the idea that when all these facts are collected it will be possible to outline some plan which will be satisfactory to the interests of the manufacturers and chemists at large and give us a uniform method of stating the specific gravity of fats and oils. This committee is working in conjunction with the Bureau of Standards, which has agreed to give it all the assistance possible along these lines.

At present there is no report to make regarding any particular methods and the committee has no recommendation to make regarding changes in methods, but expects during the coming year to make a report on a number of different subjects, including specific gravity, index of refraction, acidity, and the determination of nonfatty materials.

REPORT OF COMMITTEE ON THE STANDARDIZATION OF ALCOHOL TABLES.

The committee appointed at the last meeting of the association after considering the question of alcohol tables has the following report to make:

The Bureau of Standards has ready at the present time a complete set of tables for the determination of alcohol, based on the latest and most official data on the subject. The committee after consideration of these tables feels that it would be wise for the association to adopt them in place of those now printed in Bulletin 107, Revised.

The tables which we now have are not based on the same specific gravity of pure alcohol and we have no table from 50 per cent to 100 per cent that gives anything except full per cents. The new tables prepared by the Bureau of Standards are all based on the same specific gravity of pure alcohol, so that there is perfect agreement between them. These tables, which are submitted to the association for consideration, are five in number, as follows:

No. 1, Density of all mixtures of ethyl alcohol and water at three different temperatures, 15°/4° C., 20°/4° C., and 25°/4° C., for each per cent by weight from 0 to 100.

No. 2. Densities of mixtures of ethyl alcohol and water at 20°/4° C. for each one-tenth per cent from 0 to 100.

No. 3. Specific gravity of mixtures of ethyl alcohol and water at 60° F. (15.56°/15.56° C.) gives the per cent of alcohol by volume for each one-tenth per cent from 0 to 100.

No. 4. To convert per cents by weight, of mixtures of ethyl alcohol and water, into per cents of alcohol by volume at 60° F. for each one-tenth per cent from 0 to 100.

No. 5. A table for converting per cents by volume to per cents by weight.

In this connection, it is reported that the committee had a meeting with the Commissioner of Internal Revenue, in which the question of these tables was taken up and discussed, and it was agreed that the Bureau of Internal Revenue would adopt the standard tables proposed by the Bureau of Standards; there has been prepared for the gauger's manual a table corresponding to Table III,

at 60°/60° F., showing the degrees proof corresponding to the per cents by volume, but as yet final action has not been taken regarding this matter.

The adoption by the association of these tables would still leave us in a rather unsatisfactory position, as in a large part of the inspection work carried on under the various state laws and the national law, the use of the United States Pharmacopœia is required, and the tables recommended would not agree with those now given in that publication. It is therefore recommended that final action upon these tables be deferred a year and that the committee be instructed to take up this matter with the revision committee of the Pharmacopœia and see if it is not possible to come to some definite agreement as to the temperature at which alcohol per cents should be given, and also some agreement as to the tables to be used. It is also suggested that these tables be printed as part of the report of this committee so that they can be considered by the association during the year and be ready for adoption, if so desired, at the next meeting of the association.

(Signed) L. M. Tolman, *Chairman.*
M. E. Jaffa,
A. B. Adams,
R. J. Davidson,
H. E. Barnard,
Committee on Standardization of Alcohol Tables.

It was moved and carried that the report of the committee be accepted and printed and the committee continued. The tables to be considered by the association are printed in Circular 52, Bureau of Chemistry, U. S. Department of Agriculture, Extracts from the Proceedings of the Association for 1909, and will not be reprinted in the Proceedings pending final action.

The following motions based on recommendations made in the president's address were here introduced and carried:

(1) That the president be authorized to appoint three delegates to the U. S. Pharmacopœial Convention in 1910. [The following delegates have been appointed: Messrs. W. D. Bigelow, A. L. Winton, and E. H. Jenkins; alternates, Messrs. R. B. Fitz-Randolph, L. P. Brown, and C. E. Parker.]

(2) That a copy or abstract of referees' reports should accompany the recommendations submitted three weeks before the annual meeting of the association. [These reports are sent to the respective chairmen of Committees A, B, and C, but must subsequently be approved by the standing committee on recommendations as a whole.]

(3) That a committee be appointed by the president to compile in the form of by-laws the regulations of the association for the conduct of its business. (The president appointed Messrs. W. D. Bigelow, C. L. Penny, and R. N. Brackett.)

The following motions were then made on changes in the constitution:

(1) That section 1 of the constitution be amended by adding after the words "dairy products," in line 4, the words "human foods, medicinal plants, drugs."

(2) That section 2 of the constitution be amended to read as follows:

Section 2. Analytical chemists connected with the United States Department of Agriculture, or with any state, provincial, or national agricultural experiment station or agricultural college, or with any state, provincial, or national institution or body in North America, charged with official control of the materials named in section 1, shall alone be eligible to membership, and one such representative for each of these institutions, or boards, when properly accredited, shall be entitled to enter motions or vote in the association. Only such chemists as are connected with institutions exercising official fertilizer control shall vote on questions involving methods of analyzing fertilizers, or involving definitions, nomenclature, laws, or regulations relating to fertilizers. Only such chemists as are connected with institutions exercising official cattle-food control shall vote on questions involving methods of analyzing cattle foods or involving nomenclature, definitions, laws, or regulations relating to cattle food. Only such chemists as are connected with institutions exercising official food or drug control shall vote on questions involving methods of analyzing food or drugs or involving nomenclature, definitions, laws, or regulations relating to food or drugs. All persons eligible to membership shall become members ex officio and shall be allowed the privileges of membership at any meeting of the association after presenting proper credentials. All members of the association who lose their right to such membership by retiring from positions indicated as requisite for membership shall be entitled to become honorary members and to have all privileges of membership, save the right to hold office and vote. All analytical chemists and others interested in the objects of the association may attend its meetings and take part in its discussions, but shall not be entitled to enter motions or vote.

It was voted that these motions be referred to a committee of three, to be appointed by the president-elect and reported to the association at the next meeting. (The committee on amendments to the constitution appointed by President Withers is as follows: L. L. Van Slyke, Geneva, N. Y.; B. B. Ross, Auburn, Ala.; Wm. Frear, State College, Pa.)

THIRD DAY.

SATURDAY—MORNING SESSION.

REPORT ON ADULTERATION OF DAIRY PRODUCTS.

By J. M. Bartlett, *Referee.*

According to the recommendations made by the association at the last meeting methods for the determination of sucrose and fat in sweetened condensed milk have been studied. Material for the work was furnished by one of the leading condensed milk companies and every precaution was taken by them to have the samples uniform in composition.

Twenty-five samples were sent out to as many different chemists who had expressed a desire to cooperate in the work. The following instructions accompanied each sample:

Instructions for the Analysis of Condensed Milks.

Follow the directions given in Circular No. 43, Bureau of Chemistry, page 8, for the following: (1) Preparation of sample; (2) total solids; (3) ash; (4) protein, and (5) lactose.

Determine sucrose as follows:

Method A.—Place 25 cc of the solution used to determine lactose in a 250 cc flask, or if preferred, weigh out a new portion and clarify as for lactose. Add 175 cc of water, and 4 grams of citric acid and heat on the steam bath for forty minutes, nearly neutralize with sodium hydroxid and determine total sugars in 25 cc with Fehling's solution in the usual manner, giving the weight of cuprous oxid as well as the percentages of sucrose.

Method B.—Dilute another 25 cc portion of the solution prepared for the determination of lactose to 200 cc, add 20 cc of hydrochloric acid (38.8 per cent) and allow to stand at a temperature not below 20° C. nor above 25° C. for a period of twenty-four hours, nearly neutralize with caustic soda and determine sugar in 25 cc with Fehling's solution in the usual manner.

Determine fat as follows:

Extraction method with ether.—Prepare strips of soft white filter paper about 4 by 24 inches of the quality of the S. & S. No. 597, by soaking two or three hours in alcohol and then, after thoroughly drying in the oven, extract several hours with ether or until no residue is left from the ether as it comes through. Distribute 10 cc of a 20 per cent solution of the condensed milk carefully over the whole surface of the thoroughly dried paper. (This is best done by attaching one end of the paper to some object and holding the other end out straight so that the pipette can be emptied by passing the point back and forth over the whole surface.) To dry the paper, suspend it over a copper wire in the drying oven, where it will thoroughly dry out in two hours or much more rapidly than if coiled up or put in a tube. After drying roll up in a coil, wind with thread or small copper wire, place in the extractor, and extract for not less than eight hours. Remove the coils from the extractor, loosen the wire or thread, dry and suspend in 500 cc of water for two hours, then return the coils to the oven and dry as before, and extract again for not less than five hours. Five cubic centimeters of milk and a coil 4 by 12 inches can be used if preferred.

Gottlieb method.—Measure 10 cc of the 20 per cent solution of milk into a glass cylinder, three-fourths of an inch in diameter and about 14 inches long (a

100 cc burette or a eudiometer tube will do); add 1 cc of concentrated ammonium hydroxid and mix thoroughly with the milk; then add the following chemicals in the order given: 10 cc of 92 per cent alcohol; 25 cc of washed ether, and 25 cc of petroleum ether (boiling point not above 40° C.), the cylinder being closed with a moistened cork stopper and the contents shaken several times after the addition of each. The cylinder is then left standing for thirty minutes. The clear fat solution is next pipetted off into a small weighed flask, by means of a siphon drawn to a fine point, which is lowered into the fat solution to within 0.5 cm of the turbid bottom layer, or blown out with a wash-bottle fixture with a long adjustable delivery tube. After evaporating the ether solution in a hood the flasks are dried in a steam oven for two or three hours, and weighed. A second treatment with 10 cc of ether and petroleum ether is advisable in order to recover the last trace of fat.

Analytical Results.

Reports were received from eight laboratories, the average results agreeing very closely with those calculated from the composition of the original milk.

Average results obtained by eight collaborators on sweetened condensed milk.

Analysts.	Solids.	Ash.	Protein.	Lactose.	Sucrose.		Fat.	
					Method A.	Method B.	Double extraction.	Gottlieb method.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
E. M. Bailey, New Haven, Conn	.....	.....	.....	12.27	42.25	42.63	[a] 9.36	9.36
C. B. Morrison, New Haven, Conn	.....	.....	.....	12.06	42.18	42.51	[a] 9.36	9.30
H. D. Edmund, Storrs, Conn	74.22	1.76	7.60	.....	.....	.....	9.44	.....
B. D. Johnson, Washington, D. C	74.63	1.69	7.88	[b] 13.74	[c] 42.15	.....	9.09	9.16
W. J. Morgan, Washington, D. C	74.50	1.74	8.01	[b] 13.46	[c] 42.09	.....	9.14	9.20
J. G. Riley, Washington, D. C	75.05	1.67	7.94	[b] 14.04	[c] 42.17	.....	9.19	9.29
F. G. Seiter, Chicago, Ill	.....	.....	.....	11.71	43.40	43.45	10.07	9.44
J. M. Bartlett, Orono, Me	.....	1.73	.....	12.32	42.03	42.50	9.48	9.39
H. H. Hanson, Orono, Me	74.00	1.74	8.17	12.00	39.90	41.23	9.39	9.28
J. C. Colcord, Orono, Me	72.32	1.74	7.97	11.54	42.40	42.80	9.35	9.40
J. F. Merrill, Orono, Me	.....	.....	.....	12.00	.....	42.20	9.16	.....
A. G. Durgin, Orono, Me	73.58	1.75	.....	.....	.....	.....	9.42	.....
L. W. Fitzer, College Park, Md	73.96	1.77	7.36	12.13	44.36	44.30	9.77	.....
J. C. Reed, Amherst, Mass	73.80	1.74	8.12	12.13	42.41	43.98	9.27	.....
G. J. Second, East Lansing, Mich	.....	.....	7.71	12.04	41.90	.....	9.82	9.88
Average	74.01	1.73	7.86	12.02	42.27	42.95	9.42	9.37

[a] Babcock centrifugal method.
[b] By difference, omitted from average.
[c] Determined by polariscope.

Discussion of Results.

Comments by G. E. Patrick.—As to the method for fat in condensed milk, sweetened and unsweetened, we have in this laboratory used exclusively the Roese-Gottlieb method for three years past and believe it to be correct. The Babcock we gave up long ago as being unreliable for both kinds of condensed milk. In view of Professor Hunziker's recent claim that the Roese-Gottlieb method gives low results, in comparison with his modified extraction method, I shall during the next few months make some critical studies of both these methods.

Had it not been for Professor Hunziker's claim I should have favored the adoption of the Roese-Gottlieb method as either "official" or "provisional" at this year's meeting, but in view of his criticism and his claim of having devised a superior method, I would advise postponing action by the association for another year. We must adopt only the best method after careful study of all.

Inclosed find a copy of the Roese-Gottlieb method as we use it here, which you are at liberty to use in your report if you see fit. As to the boiling point of the petroleum ether, we prefer it from 40° to 60° C., or at the highest not above 70° C., as above this point too much time is required to expel it from the

fat and at below 40° it is apt to cause trouble by spontaneous boiling during the summer months in Washington. But the solvent power, under the conditions of the method, appears to be the same for a petroleum ether of all these boiling points and even up to 80° C. We find no advantage in washing the ethyl ether; 95 per cent alcohol by volume is satisfactory.

The Roese-Gottlieb method for the determination of fat in condensed milk (as practiced at the Dairy Laboratory, Bureau of Chemistry).

Prepare a water solution of exactly known content—not above 40 per cent and not below 20 per cent—of a portion of the smooth homogeneous sample. Of this solution measure 10 cc, or weigh correctly about 10 grams, into a Rohrig tube,[a] or a glass cylinder three-fourths of an inch in diameter and about 14 inches high, to which a narrow siphon can be fitted[b] or other similar apparatus add 1 cc of concentrated ammonium hydroxid (2 cc if the sample is sour) and mix thoroughly with the milk. Add 10 cc of alcohol, 95 per cent by volume, and shake well. Then add 25 cc of ethyl ether, shake *vigorously* for two or three minutes, add 25 cc of petroleum ether (boiling point below 70° C. preferably), and shake again. Let stand fifteen minutes or until the upper liquid is clear and its lower level constant.

Should the dividing line not be distinct add 0.3 gram of powdered sodium chlorid and agitate sufficiently to dissolve the salt; this will usually give a sharp dividing line.

Take the upper and lower readings of the ethereal liquid and then draw off as much as possible—usually 0.5 to 0.8 cc is left—into a weighed flask through a diminutive filter, which must then be washed with a few cubic centimeters of the mixed ethers (1:1). Read the volume of ethereal liquid left in the tube and deduct from the total volume.

Evaporate the drawn off and filtered liquid slowly in a hood and then dry in a boiling-water oven, one hour or less at a time, until loss of weight ceases. Extract the liquid remaining in the tube in the same manner as before, but this time, for economy, the volume of the ethers used may be reduced to 10 to 15 cc of each—equal volumes always in each case. It is preferable to evaporate and weigh this extract separately, as a check upon the work. The ether used must be tested for residue upon evaporation, and if any is found the results of analysis must be corrected therefor.

The purity of the dried and weighed fats should always be proved by dissolving in a little petroleum ether; should a residue be found (which can only happen when a trace of the aqueous liquid has accidentally passed the filter) it must be washed in the flask, dried, and its weight deducted from that of the crude fat.

This method is equally applicable to sweetened and unsweetened condensed milks, to ice cream, cream, milk, skim milk, buttermilk, and whey. With substances of low fat content the second extraction may be omitted, in which case the weight of the fat actually obtained must be increased to correspond to the entire volume of ethereal liquid measured in the tube.

Comments by referee.—So few laboratories have cooperated this year that it is not thought best to draw any definite conclusions from the figures obtained. The results on fat determinations by the double-extraction method are quite satisfactory and are, perhaps, as good as can be expected from different men working under varying conditions. The Gottlieb method in most instances gives comparable results; though slightly lower than those by the double-extraction method, they are sufficiently accurate for commercial purposes. The Babcock method, with the Leach or Farrington modifications, when handled by an expert, will give good results on sweetened milks that clear up well on the addition of copper sulphate. It often happens, however, with some goods, that the copper does not give a clear and clean separation, and in such cases low results are obtained.

Early in the summer Bulletin 134 of the Indiana Agricultural Experiment Station, which contains a very careful and thorough investigation of the

[a] Zts. Nahr. Genussm., 1905, **9**: 531. [b] Landw. Vers. Sta., 1892, **40**: 6.

methods of determining fat in unsweetened condensed milk, was published. Manufacturers have for some time known that there was an apparent loss of fat in the process of condensing, and chemists could not find as much fat in the finished product as went in with the milk. Various theories were advanced to account for this loss, but no reasonable explanation could be given why there should be a loss of butter fat at the temperatures that were used in condensing. The work of Professors Hunziker and Spitzer has thrown some light on the subject and demonstrated that the unsweetened condensed milk presents fully as many difficulties in the estimation of fat as the sweetened product. The modifications of the ether extraction and the Babcock methods brought out by them are worthy of trial, and if adaptable to both kinds of milk would obviate the necessity of adopting two methods.

The figures on the determination of lactose are not as satisfactory as one could wish, but when it is considered that the conditions under which the work is done very materially affect the results, the variations are not surprising. The determinations of sucrose are more satisfactory than those for lactose, and with one or two exceptions are as close as could be expected for so high a percentage. The method for inversion with citric acid seems very satisfactory and takes less time than the method using hydrochloric acid in the cold.

The work of Watts and Tempany [a] shows that a 3 per cent solution of citric acid is without inverting effect on lactose even when boiled for a considerable length of time. The sucrose is practically all inverted by forty minutes' boiling in a 3 per cent solution.

Recommendations.

It is recommended—

(1) That the methods for the analysis of condensed milk presented to the association at its last meeting and referred to the referee for 1909 be adopted as official. These methods are given in the Proceedings of the Association for 1908, page 158, and Circular 43, page 8, of the Bureau of Chemistry, and are for (1) Preparation of sample, (2) total solids, (3) ash, and (4) lactose.

(2) That the method for inverting sucrose in the sweetened product with citric acid, using 3 per cent of the acid and boiling for forty minutes, be again tested.

(3) That methods for determining fat in both sweetened and unsweetened condensed milk be studied, giving special attention to those modifications of the Babcock and extraction methods recently brought out in Bulletin 134 of the Indiana Agricultural Experiment Station.

(4) That the standard for Babcock glassware, proposed by E. B. Holland and referred by vote of the association to the referee for 1909, be adopted as the official standard. (See Bulletin 122, p. 189; also Circular 43, p. 9, Bureau of Chemistry.)

OCCURRENCE OF METHYL PENTOSAN IN CATTLE FOODS.

By Fred W. Morse, *Associate Referee.*

The associate referee has continued the work of last year on the solubility in alcohol of the precipitate of furfural phloroglucid obtained in the determination of pentosans in cattle foods. Both wheat bran and corn stover were

[a] The Analyst, 1905, **30**: 119.

studied because they are both known to yield considerable furfural, and one would represent the grains and the other the coarse fodders. Rock weed (*Fucus vesiculosis*) was also employed because it is known to yield methyl-furfural together with furfural.

Repeated quantitative tests were made by W. L. Adams on bran by Ellett's method [a] of successive extraction with hot alcohol. Besides 95 per cent alcohol, strengths of 70 per cent and 60 per cent were used for comparison. Several partial distillations were made also to determine whether the solubility would be proportional to the precipitate, or whether methyl-furfural concentrated in the early or later portions.

The results of these various tests are summarized in the following table, and it may be noted that there is very little solubility under any of the conditions, and it may well be due to the solubility of furfural-phloroglucid itself, which has always to be considered in calculating the percentage of pentosans.

Solubility of the phloroglucids obtained from wheat bran.

[Two grams used in each case.]

Weight of precipitate.[a]	Amount dissolved in—		
	95 per cent alcohol.	70 per cent alcohol.	60 per cent alcohol.
Gram.	*Gram.*	*Gram.*	*Gram.*
0.4417 (a)	0.0099		
.3604 (b)		0.0040	
.3687 (b)			.0059
After partial distillation:			
0.1030 (a)	.0018		
.0893 (a)	.0023		
.2303 (b)		.0022	
.2173 (b)		.0058	
.2994 (b)			.0025
.2073 (b)			.0042

[a] (a) and (b) represent two different lots of bran.

Qualitative tests alone were used with corn stover, and it was invariably found that the first two portions of alcohol used for extracting the precipitate were colored; but the coloration was little if any deeper than in the bran extracts, so weighings were not made.

Efforts were made to identify methyl-furfural in the distillates from the bran and the corn stover. The tests described by Widtsoe and Tollens [b] failed to yield any evidence of its presence; but De Chalmot's test [c] after several trials showed slight evidences of its presence in the bran. Since rockweed was known to yield both furfural and methyl-furfural, mixtures of bran and rockweed of varying proportions were tried.

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[a] Inaug. Dissertation, Göttingen, 1904; Ber. d. chem. Ges., 1905, **38**: 492.

[b] Ber. d. chem. Ges., 1900, **33**: 144–146.

[c] Amer. Chem. J., 1893, **15**: 278.

Solubility of methyl-furfural in mixtures of bran and rockweed.

Description of sample.	Precipitate.	Amount dissolved in—	
		70 per cent alcohol.	60 per cent alcohol.
	Gram.	*Gram.*	*Gram.*
2 grams of bran	0.3604	0.0040	
	.3687		0.0059
1.5 grams of bran, 0.5 grams of rockweed	.3397	.0107	
	.3355		.0096
1 gram of bran, 1 gram of rockweed	.3155	.0223	
	.3159		.0060
0.5 gram of bran, 1.5 grams of rockweed	.2833	.0266	
	.2984		.0130
2 grams of rockweed	[a] .2487	[a] .0433	

[a] Mean of several determinations using 95 per cent alcohol.

These results furnished corroborative evidence that the solubility of the bran precipitate is mainly due to furfural phloroglucid, by the marked difference in the effects of 70 per cent alcohol and 60 per cent on the rockweed mixtures, because of the greater solubility of methyl-furfural phloroglucid in the stronger alcohol, while the furfural phloroglucid is about equally affected. Messrs. Woll and Brannon, of Wisconsin, made solubility tests on the following materials:

Phloroglucid precipitate soluble in 95 per cent alcohol.

Samples.	Weight of precipitate from 1 gram.	Weight of precipitate in 95 per cent alcohol.	Samples.	Weight of precipitate from 1 gram.	Weight of precipitate in 95 per cent alcohol.
	Gram.	*Gram.*		*Gram.*	*Gram.*
Washed wheat bran	0.4374	0.0098	Oat straw	0.2717	0.0064
Ground rice	.0086	.0038	Corn fodder	.2284	.0086
Wheat straw	.2240	.0072	Gluten feed	.1798	.0110

These results as a whole indicate that while methyl pentosans may exist, and probably do, in our common cattle foods, they are present in traces only and the provisional method of pentosan determination needs no modification for practical use.

REPORT ON SUGAR.

By A. Hugh Bryan, *Referee*, and H. P. Agee, *Associate Referee*.

The collaborative work of this year consisted of three lines of investigation: (1) Methods of moisture determination; (2) determination of reducing sugars; and (3) effect of clarifying agents on polarization.

Two samples were sent out, one of a low-grade Louisiana sugar and the other a residue cane molasses, with the following instructions:

Instructions for Cooperative Work.

MOISTURE.

The determination is to be made on both samples.

(1) Dry 2 grams of material on sand to constant weight in a vacuum oven at 70° C. Add water to dissolve the sugar and to obtain a good mixture with the sand.

(2) Dry 2 grams of material on sand in water-jacketed oven for ten consecutive hours.

(3) Horne's method (Bulletin 116, Bureau of Chemistry, p. 23). Weigh 1 gram of molasses into a flat-bottom dish 3 inches wide and 0.5 inch deep and containing a glass rod. Mix about 0.8 cc of water well with the molasses, and then weigh carefully about 30 grams of dry quartz sand, previously extracted with hydrochloric acid, and add to the diluted molasses. Place the dish on an open boiling-water bath and stir the contents carefully and frequently during half an hour. Then place the dish in a water-jacketed air bath where it is heated at the temperature of boiling water for two hours. After cooling weigh and reheat for one-hour intervals until the weight is constant.

(4) By the Brix spindle. See Areometric method (1), page 65, Bulletin 107, Revised.

(5) By the refractometer. Obtain the refractive index of the molasses, and also make a solution of equal weights of molasses and water and obtain its index. With the sugar dissolve as in the half dilution of molasses. By means of the table of Geerligs (Bulletin 122, page 169) obtain the percentage of dry substance; this subtracted from 100 gives the per cent of moisture.

DETERMINATION OF REDUCING SUGARS.

Determine the reducing sugars by either Allihn's method (Bulletin 107, Revised, page 49) or Munson and Walker's method (Ibid., page 241), on a solution clarified with neutral lead acetate.

(1) Weigh the precipitated cuprous oxid as such and calculate to dextrose.

(2) The crucible from (1) is to be put in muffle and heated to oxidize the cuprous oxid to cupric oxid. Calculate the cupric oxid to dextrose. In the muffle at the same time place two crucibles that have been weighed as in paragraph (1) but have not been used to filter any of the solution. Obtain their weight after heating, noting the loss in weight, if any.

(3) The crucibles from (2) are treated with hot nitric acid (1:1) to dissolve all of the cupric oxid and the copper is titrated by Low's method (Bul. No. 107, Revised, page 241).

If time admits, the same line of procedure can be tried, using basic lead acetate as a clarifying agent instead of neutral lead.

Polarization.

Weigh out a normal weight and make up to 100 cc, or to such a multiple thereof as may be necessary to secure an accurate polarization after clarifying as follows:

(1) With lead subacetate solution (Bul. 46, pages 38–39; also Bul. 107, page 40).

(2) With normal lead acetate solution (saturated solution of lead acetate in water).

(3) With Horne's dry lead subacetate (J. Amer. Chem. Soc., 1904, **26**: 186).

(4) With Herles' solution: No. 1, 250 grams of lead nitrate to 500 cc of water; No. 2, 25 grams of sodium hydroxid to 500 cc of water. Use equal parts of the two solutions for clarification.

(5) Repeat the preceding four tests, increasing the amount of the clarifying agent.

(6) Invert portions of all the solutions by the first or second method (Bul. 107, page 41), and determine the invert reading. To remove lead before inversion neutral potassium oxalate or dry sodium carbonate can be used. With the latter reagent the solution should be neutralized with acetic acid before adding the 5 cc of hydrochloric acid. Sucrose is calculated from the formula:

$$\text{Sucrose} = \frac{a-b}{142.66 - \frac{t}{2}}$$

In these polarizations record all temperatures of polarization, dilutions, etc., that the results may be compared upon as uniform a basis as possible.

It is also urged that the work on the samples be begun immediately upon their arrival to avoid changes in composition which might result from fermentation.

A number of chemists signified their willingness to cooperate, and reports, in whole or in part, were received from most of them.

Moisture Determination.

As is noted in the instructions, the object of this study was to compare the official, the vacuum, and Horne's methods with the refractometer method. The results obtained by the collaborators are found in Table 1.

Table 1.—*Moisture determinations.*

Analyst and sample.	Vacuum method.		Official method.		Horne's method.		Refractometer.		Spindle brix.
	Time.	Per cent.	Time.	Per cent.	Time.	Per cent.	Undiluted.	Half diluted.	
Sugar sample:	*Hrs.*		*Hrs.*		*Hrs.*		*P. ct.*	*P. ct.*	*P. ct.*
H. C. Gore, Washington, D. C	.....	1.08	10	1.27	.....	.....	.....	.....	.....
W. D. Horne, Yonkers, N. Y	.....	1.45	6	1.62	.....	.....	.....	.....	.....
Do	.....	.....	9	1.62	.....	.....	.....	.....	.....
C. I. Lott, Chicago, Ill	.....	.....	10	1.48	3	1.06	.....	2.45	.....
S. F. Sherwood, Washington, D.C	16	1.47	10	[a][b].86	3	1.37	.....	.....	.....
W. D. Taggart, New Orleans, La	.....	1.59	.....	1.50	.....	1.60	.....	2.00	.....
P. H. Wessels, Kingston, R. I	.....	[b]c.60	10	[b]d.80	.....	1.50	.....	1.85	.....
F. G. Smith, St. Paul, Minn	.....	.....	10	1.40	.....	.....	.....	1.88	.....
Average	.....	1.40	.....	1.48	.....	1.38	.....	.....	.....
Molasses sample:									
H. C. Gore, Washington, D. C	.....	22.63	10	25.03	.....	23.97	21.81	21.69	.....
W. D. Horne, Yonkers, N. Y	.....	24.00	10	24.14	4	24.07	.....	.....	.....
C. I. Lott, Chicago, Ill	.....	.....	10	23.74	3	23.57	21.81	21.65	.....
S. F. Sherwood, Washington, D.C.	16	22.63	10	24.12	4	23.41	22.50	22.06	18.60
F. G. Smith, St. Paul, Minn	.....	.....	.....	23.80	.....	24.40	21.64	21.66	[e]18.30
W. D. Taggart, New Orleans, La	.....	24.31	.....	24.08	.....	25.35	.....	22.39	23.56
P. H. Wessels, Kingston, R. I	17	21.48	10	24.28	.....	.....	.....	.....	.....
Average	.....	23.01	.....	24.06	.....	24.13	21.94	21.89	.....

[a] 1.79 after twenty hours.
[b] Not included in average.
[c] 1.28 with no water added.
[d] 1.49 heated one hour more.
[e] From half-diluted solution.

The average figures show that the official method gives the highest percentage of moisture. In the case of the sugar, Horne's method and the vacuum method give comparable figures, and are lower than the official method. The refractometer method is hardly fitted for moisture in sugar. The results of individuals on the separate determinations vary considerably; this variation can hardly be due altogether to variation in the sample under examination.

With the molasses sample, Horne's method gives higher results than the other methods. The individual determinations in the official method show the closest agreement, excluding one result. Horne's method shows a fair agreement.

The refractometer method gives closely agreeing results in the hands of different chemists. By Horne's method the results are obtained in from three to four hours, but the manipulation is rather trying and precludes many determinations a day. Stirring on the water bath takes time also, and large dishes are needed to prevent loss of the sample. No comments were made by the collaborators on these methods.

The subject of moisture or dry substance determination is a very important one and should be further studied. Some German sugar chemists have shown that a relation should exist between the quantity of sand used and the quantity of material dried. Koydl[a] shows that the size of the sand particles

[a] Centrbl. Zucker-Ind., 1909, **17**: 1064–1065.

used plays an important part, as he obtained 69.79 per cent of dry substance when drying on large-grain sand and 71.14 per cent when drying on small-grain sand. This investigator favors the use of granulated sugar as a medium on which to dry sugar solutions in preference to sand, his results showing 71.77 per cent and 71.80 per cent of dry substance on sugar, and 70.99 per cent and 71.02 per cent on sand. These points should have the attention of the referee next year, especially the question of the size and quantity of sand to be used.

The refractometer method was adopted provisionally in 1908. No mention was made of the method to be pursued in the case of dark-colored products. Under such a condition a reading is difficult on account of the blurred dividing line. Most observers would remedy this by diluting the molasses with an equal weight or volume of water and determining the refractive index of this solution, but such a procedure would lead to error, as on the addition of water there is a contraction in volume, and the coefficients of the various nonsugars and salts differ from each other and from those of sugar and the contraction coefficients also vary. As a consequence, the gravity and refractive index of the diluted solution is not half that of the original solution. To reduce these variations of contraction to the minimum a concentrated pure sugar solution is used as a diluent.

TABLE 2.—*Refractometer determination of dry substance on diluted and undiluted samples.*

Number of sample.	Undiluted.	Diluted half.	
		Water.	Sugar solution.
1	80.57	83.24	80.91
2	72.32	72.94	72.21
3	77.92	78.44	77.91
4	73.92	75.34	73.81
5	82.05	84.44	82.41

All of these are cane molasses and in the undiluted form could be easily read. The results on the half dilution with water are from 0.62 to 2.7 per cent higher than on the undiluted, while the half dilution with sugar solution varies only from 0.0 to 0.3 per cent.

Tischtschenko [a] calls attention to this possible error in the determination and recommends the use of a pure sugar solution, and Von Lippman [b] corroborates these results. It seems well established therefore that a sugar solution should be used in diluting dark-colored solutions in preference to water. The formula for calculating the dry substance when using a concentrated sugar solution as a diluent is—

$$X=\frac{(A+B)C-BD}{A}$$

in which X=the per cent of dry substance of the original sample; A, the number of grams of the original substance mixed with B (the grams of concentrated pure sugar solution); C, the per cent of dry substance of the mixture obtained from its refractive index, and D=the per cent of dry substance of

[a] Zts. Ver. d. Zucker-Ind., February, 1909, p. 103.

[b] Deutsche Zucker-Ind., 1909, 34: 401.

the pure sugar solution obtained from its refractive index. The method of procedure is simply to prepare a concentrated granulated sugar solution, mix a weighed quantity of this in a small beaker with a weighed quantity of the original solution or sample, and determine the refractive index of the mixture.

Reducing Sugar Determination.

Collaborative work.

The object of this study was to note the difference in the percentage of reducing sugars obtained when determining the copper precipitate by weighing as red oxid, as black oxid, and by titrating the copper by Low's method. Incidentally the effect of basic lead acetate as compared with the neutral lead was shown. The results of the collaboration are given in Table 3.

Table 3.—*Reducing sugars as dextrose in sugar and molasses by three methods.*

Analyst and character of sample.	Neutral lead acetate.			Basic lead acetate.		
	Weighed as—		By Low's method.	Weighed as—		By Low's method.
	Cuprous oxid.	Cupric oxid.		Cuprous oxid.	Cupric oxid.	
Sugar sample:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
A. Hugh Bryan, Washington, D. C.	2.88	2.83	2.79	2.69	2.63	2.60
W. D. Horne, Yonkers, N. Y.	2.75	2.65	2.65	2.81	2.71	2.71
C. I. Lott, Chicago, Ill.	2.66	2.56	.....	2.49	2.41	.....
S. F. Sherwood, Washington, D. C.	2.68	2.59	2.57	2.60	2.52	2.49
F. G. Smith, St. Paul, Minn.	2.32	2.28	2.30	.....	.....	.....
W. G. Taggart, New Orleans, La.	3.12	2.99	2.90	.....	.....	.....
P. H. Wessels, Kingston, R. I.	3.30	3.25	3.30	.....	.....	.....
Average	2.82	2.73	2.75	2.65	2.57	2.60
Molasses sample:						
A. Hugh Bryan, Washington, D. C.	36.16	35.73	34.32	33.64	33.27	33.54
R. B. Blanchard, St. Croix, D. W. I.	36.52	36.30	.....	.....	.....	.....
C. I. Lott, Chicago, Ill.	36.34	36.00	.....	34.96	34.68	.....
S. F. Sherwood, Washington, D. C.	35.30	34.83	34.46	34.71	34.31	33.87
F. G. Smith, St. Paul, Minn.	34.53	34.41	34.48	.....	.....	.....
W. G. Taggart, New Orleans, La.	35.41	35.24	35.32	.....	.....	.....
P. H. Wessels, Kingston, R. I.	35.01	34.07	34.89	.....	.....	.....
Average	35.61	35.22	34.69	34.44	34.09	33.71

The average results show, in the case of sugar, a fair agreement between the percentages, whether determined from the weight of cuprous or of cupric oxid, or by Low's method. The weighing as cuprous oxid, however, gives the highest results. In the case of molasses, the results of weighing the red oxid (cuprous oxid) are very much higher than Low's method and slightly higher than the weighing as cupric or black oxid. This indicates that the precipitation of the cuprous oxid brings down with it some mineral matter, and such is the case as the strongly alkaline Fehling solution precipitates some of the salts of the molasses added. For correct results, the estimation of the copper in the precipitate by some such method as Low's is necessary. This subject is further discussed on page 181, and a recommendation made concerning it. The table also shows the difference between the results obtained by neutral lead acetate and by basic lead acetate as clarifying agents, the latter removing quite a percentage of the reducing sugars.

S. F. Sherwood and the referee determined the reducing sugars in the solution used for polarization with the following results:

TABLE 4.—*Reducing sugars precipitated by the various clarifying agents.*

Sample and clarification agent.	A. H. Bryan.		S. F. Sherwood.		Average per cent of dextrose removed by clarifier.
	Dextrose.		Dextrose.		
	Per cent present.	Per cent removed by clarifier.	Per cent present.	Per cent removed by clarifier.	
Sugar sample:					
Neutral lead acetate	2.88		2.68		
Neutral lead acetate in excess	2.83	1.74	2.62	2.24	1.99
Lead subacetate	2.73	5.21	2.60	2.98	4.10
Lead subacetate in excess	2.69	6.60	2.50	6.72	6.66
Dry lead subacetate	2.80	2.78	2.61	2.62	2.70
Dry lead subacetate in excess	2.72	5.55	2.52	5.97	5.76
Basic lead nitrate	2.74	4.86	2.52	5.97	5.42
Basic lead nitrate in excess	2.69	6.60	2.45	8.58	7.59
Molasses sample:					
Neutral lead acetate	36.16		35.30		
Neutral lead acetate in excess	35.70	1.27	34.74	1.59	1.43
Lead subacetate	34.80	3.76	34.71	1.67	2.72
Lead subacetate in excess	33.60	7.08	33.54	4.99	6.04
Dry lead subacetate	35.28	2.43	34.74	1.67	2.05
Dry lead subacetate in excess	33.46	7.47	33.43	5.30	6.38
Basic lead nitrate	35.14	2.82	34.09	3.43	3.12
Basic lead nitrate in excess	33.85	6.39	32.15	8.92	7.65

The table shows the per cent of dextrose removed by the clarifying agents based on the percentage of reducing sugars determined with the necessary amount of neutral lead acetate as the correct figure. The data prove that any excess of the various precipitants should be carefully avoided, as it produces a marked effect.

SPECIAL STUDY.

In accordance with the instructions of the committee on recommendations adopted in 1908, a special study was made by the referee and S. F. Sherwood to determine what limitation should be placed on the method of determining reducing sugars by weighing the cuprous oxid precipitated.

As has been shown by Browne in his reports as referee on sugar for 1906 and 1907, the precipitate of cuprous oxid in molasses and sugar solutions contains considerable quantities of other substances and, therefore, weighing this precipitate direct will give too high results. The work of the collaborators on this year's samples also show this fact. In order to determine whether such a condition obtained generally in the case of food products, various foods were examined and the results calculated to per cent of invert sugar, the following three procedures being followed: (1) To weigh as the cuprous oxid; (2) to place this crucible in a muffle and burn to the cupric oxid and weigh again, and (3) to dissolve this cupric oxid in hot nitric acid and determine the copper by Low's method. The results are given in Table 5.

TABLE 5.—*Determination of reducing sugars in various food products by three methods.*

Substance.	Invert sugar.			Substance.	Invert sugar.		
	Weighed as—		By Low's method.		Weighed as—		By Low's method.
	Cuprous oxid.	Cupric oxid.			Cuprous oxid.	Cupric oxid.	
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
Calves-foot jelly	2.29	2.11	2.20	Maple sirups	32.09	31.65	31.90
Current jelly	49.35	48.45	49.15	Do	2.65	2.25	2.60
Raspberry jelly	38.05	37.60	37.44	Cane sugars	2.64	2.57	2.54
Plum jelly	34.16	33.56	32.31	Do	2.73	2.64	2.61
Apple butter	28.05	27.68	27.67	Do	5.28	5.00	5.01
Apple butter containing glucose	28.47	28.17	28.00	Do	6.82	6.63	6.45
Apricot butter	28.54	28.38	27.43	Cane molasses	35.07	33.42	32.41
Preserved strawberries	52.98	52.40	52.60	Do	19.44	18.89	18.94
Blackberry jam	52.93	52.05	51.70	Do	18.05	17.39	17.12
Honey	72.31	71.95	72.02	Do	17.60	17.32	17.27
Do	67.09	66.75	66.88	Malt extract	36.91	.....	33.86
Do	70.31	69.81	69.95	Do	35.10	.....	32.60
Do	74.20	73.81	74.02	Beer	2.09	.....	2.08
Maple sirups	2.07	1.92	2.00	Do	2.06	.....	2.07
Do	1.26	1.18	1.21	Pure dextrose	95.84	.....	95.82
				Do	94.97	.....	94.95
				Do	97.25	.....	97.28

It is seen that in nearly all cases the per cent of invert sugar when using Low's method for determining the copper in the precipitate is lower than when weighing as cuprous oxid. The exceptions are pure dextrose, beer, and a few determinations on some of the other products. Molasses, malt extracts, and some of the jellies and jams show large differences, while in honeys and maple sirups they are slight. Considering the fact that so many show a difference, and quite a large one in some cases, it seems that the method of weighing as red or cuprous oxid should be limited to pure sugar solutions, while in most foods and other products the copper should be determined in the precipitate direct by some such method as Low's.

EFFECT OF CLARIFYING AGENTS ON POLARIZATION.

This investigation is a continuation of work that has been under way for several years. The clarifying agents were basic lead acetate, both in solution and dry, neutral lead acetate in solution, and basic lead nitrate. Hydrosulphite was not tested as it has been found to give too low results for direct polarization. The cooperative results are given in tabular form. In the first four columns are the figures reported as obtained with the necessary amount of clarifier, but some of these results should be included under the "Excess amount of clarifier" as the quantities used were far too large.

TABLE 6.—*Polarization of sugar, using different clarifying agents.*

[Normal weight to 100 cc; polarized in a 200-mm tube; sucrose factor 142.66.]

Analyst and clarifying agent.	Necessary amount of clarifier.				Excess amount of clarifier.			
	Amount of clarifying agent.	Direct polarization.	Temperature of polarization.	Sucrose (Clerget).	Amount of clarifying agent.	Direct polarization.	Temperature of polarization.	Sucrose (Clerget).
	cc.	*° V.*	*° C.*	*Per ct.*	*cc.*	*° V.*	*° C.*	*P. ct.*
Lead subacetate solution:								
H. P. Agee, New Orleans, La	2	90.61	28	92.85	.....	.....	.....	.....
A. H. Bryan, Washington, D. C	1.5	90.80	20	93.24	3	90.90	20	93.23
G. H. Hardin, New York, N. Y	1	90.70	20.5	93.33	.....	.....	.....	.....
W. D. Horne, Yonkers, N. Y	[a] 2	90.60	.....	91.95	[a] 4	90.75	.....	92.03
W. L. Howells, New Orleans, La	1	91.10	22.0	92.20	3	91.00	27	92.28
C. I. Lott, Chicago, Ill	4	91.10	24	91.77	.....	.....	.....	.....
S. F. Sherwood, Washington, D. C	1.5	90.75	20	92.71	3	90.90	20	91.14
F. G. Smith, St. Paul, Minn	1.5	90.90	20	91.82	3	91.05	20	92.07
W. G. Taggart, New Orleans, La	1	90.70	27	93.14	2	90.80	27	93.12
M. H. Wiley, New York, N. Y	1	90.85	21	93.54	.....	.....	.....	.....
Average	.....	90.80	.....	92.65	.....	90.90	.....	92.31
Neutral lead acetate:								
H. P. Agee, New Orleans, La	1	90.67	28	92.95	.....	.....	.....	.....
A. H. Bryan, Washington, D. C	[b] 2	90.75	20	93.03	[b] 4	90.80	20	93.07
G. H. Hardin, New York, N. Y	2	90.70	20.5	93.21	.....	.....	.....	.....
W. D. Horne, Yonkers, N. Y	[c] 3.6	90.65	.....	91.55	.....	.....	.....	.....
W. L. Howells, New Orleans, La	[b] 1	91.00	22.0	92.07	[b] 3	91.10	22	91.98
C. I. Lott, Chicago, Ill	3	91.10	24	92.07	.....	.....	.....	.....
S. F. Sherwood, Washington, D. C	[b] 2	90.90	20	92.82	[b] 4	90.90	20	91.57
F. G. Smith, St. Paul, Minn	1.5	90.50	20	91.30	3	90.75	20	91.85
W. G. Taggart, New Orleans, La	2	90.70	27	93.04	4	90.70	27	93.04
M. H. Wiley, New York, N. Y	2	90.75	21	93.43	.....	.....	.....	.....
Average	.....	90.77	.....	92.44	.....	90.85	.....	92.30
Dry lead subacetate:	*Grams.*				*Grams.*			
H. P. Agee, New Orleans, La	.....	90.63	28	92.86	.....	.....	.....	.....
A. H. Bryan, Washington, D. C	0.5	90.55	20	92.39	1.0	90.60	20	92.42
G. H. Hardin, New York, N. Y	.5	90.55	21.5	93.17	.....	.....	.....	.....
W. D. Horne, Yonkers, N. Y	.27	90.50	.....	.....	.....	.....	.....	.....
W. L. Howells, New Orleans, La	1.00	90.80	22	91.90	2.0	90.90	22	92.07
C. I. Lott, Chicago, Ill	.97	91.56	24	92.27	1.25	91.50	24	92.07
S. F. Sherwood, Washington, D. C	.5	90.70	20	92.67	1.00	90.60	20	91.35
W. G. Taggart, New Orleans, La	.....	90.80	27	93.20	.....	90.70	27	93.20
M. H. Wiley, New York, N. Y	.5	90.60	21	93.27	.....	.....	.....	.....
Average	.....	90.74	.....	92.72	.....	90.86	.....	92.22
Basic lead nitrate:	*cc.*				*cc.*			
H. P. Agee, New Orleans, La	1	90.64	28	92.92	.....	.....	.....	.....
A. H. Bryan, Washington, D. C	2	90.65	20	92.87	4	90.80	20	92.90
G. H. Hardin, New York, N. Y	2	90.75	20.5	93.25	.....	.....	.....	.....
W. D. Horne, Yonkers, N. Y	1.44	90.75	.....	.....	.....	.....	.....	.....
W. L. Howells, New Orleans, La	2	91.15	22.0	92.17	6	91.20	22	92.28
C. I. Lott, Chicago, Ill	5	91.20	24	91.99	.....	.....	.....	.....
S. F. Sherwood, Washington, D. C	2	90.90	20	92.82	4	90.90	20	91.14
F. G. Smith, St. Paul, Minn	1.5	90.82	20	91.76	3	90.88	.....	92.02
W. G. Taggart, New Orleans, La	1	90.70	27	93.14	2	90.70	27	93.28
M. H. Wiley, New York, N. Y	2	90.75	21	93.50	.....	.....	.....	.....
Average	.....	90.83	.....	92.71	.....	90.89	.....	92.32

[a] 24° Brix. [b] Saturated solution. [c] 10 per cent solution.

The average results on the direct polarization of the sugar from each clarification agree very closely, the range of the extremes being only 0.09°, which is well within the limits of error. However, individual polarizations under each clarifier vary much more, sometimes as much as 1°. When using an excess of clarifying agent, the average direct polarizations vary only 0.05°. Adding an excess raises the direct polarization in each case and lowers the Clerget sucrose; in the latter determination the individual variation is very large, probably due to different methods of inversion. The average results on the Clerget sucrose vary 0.10 per cent with the excess of clarifier and 0.28 per cent with the necessary amount.

The molasses sample was particularly suited to testing the effect of clarifying agents. It was a typical Louisiana black-strap molasses, very thick and black. The normal weight in 100 cc or in 200 cc would hardly give a light enough solution, although some of the collaborators used the latter dilution. Half-normal weight in 200 cc gave a solution very easily read, but with this dilution an error of 0.1° on the reading would give a 0.4° error in the final result.

TABLE 7.—*Polarizations of molasses with different clarifying agents.*

[Half normal weight to 200 cc, polarized in 200 mm tube; sucrose factor 142.66.]

Analyst and clarifying agent.	Necessary amount of clarifier.				Excess amount of clarifier.			
	Amount added.	Direct polarization.	Temperature of polarization.	Sucrose (Clerget).	Amount added.	Direct polarization.	Temperature of polarization.	Sucrose (Clerget).
	cc.	*° V.*	*° C.*	*P. ct.*	*cc.*	*° V.*	*° C.*	*P. ct.*
Lead subacetate solution:								
H. P. Agee, New Orleans, La	5	12.00	30	22.15	.....	.....	.....	.....
R. P. Blackwood, St. Croix, D. W. I	6	12.60	31	20.29	.....	.....	.....	.....
A. Hugh Bryan, Washington, D. C	8	13.20	20	22.46	14	13.60	20	23.52
G. H. Hardin, New York, N. Y	8	12.20	24	23.48	.....	.....	.....	.....
W. D. Horne, Yonkers, N. Y	[a] 11	12.00	22	23.69	.....	.....	.....	.....
W. L. Howells, New Orleans, La	14	13.20	28	23.62	20	14.00	28	24.09
C. I. Lott, Chicago, Ill	[b] 40	14.20	24	23.11	.....	.....	.....	.....
S. F. Sherwood, Washington, D. C	6	12.20	20	22.46	12	13.60	20	22.52
F. G. Smith, St. Paul, Minn	10	12.40	25	23.36	20	12.40	25	23.05
W. G. Taggart, New Orleans, La	6	11.60	23	21.93	8	11.60	23	22.26
M. H. Wiley, New York, N. Y	8	12.20	24	23.14	.....	.....	.....	.....
Average	.....	12.53	.....	22.70	.....	13.04	.....	23.08
Neutral lead acetate:								
H. P. Agee, New Orleans, La	8	12.00	30	21.98	.....	.....	.....	.....
R. P. Blackwood, St. Croix, D. W. I	8	11.84	31.8	21.96	.....	.....	.....	.....
A. Hugh Bryan, Washington, D. C	[c] 25	12.00	20	23.47	40	12.40	20	24.27
G. H. Hardin, New York, N. Y	[c] 25	12.40	24	23.25	.....	.....	.....	.....
W. D. Horne, Yonkers, N. Y	[d] 10	10.40	23	21.95	[d] 16	10.90	23	22.84
W. L. Howells, New Orleans, La	20	13.20	28	24.25	.....	.....	.....	.....
C. I. Lott, Chicago, Ill	[c] 30	11.60	22	21.27	.....	.....	.....	.....
S. F. Sherwood, Washington, D. C	[c] 8	12.20	20	21.80	16	12.00	20	22.31
F. G. Smith, St. Paul, Minn	10	12.40	25	23.05	20	12.40	25	23.05
W. G. Taggart, New Orleans, La	8	11.20	23	21.62	16	11.20	23	21.62
M. H. Wiley, New York, N. Y	[c] 25	11.80	24	22.80	.....	.....	.....	.....
Average	.....	11.84	.....	22.49	.....	11.78	.....	22.75
Dry lead subacetate:	*grams.*				*gms.*			
H. P. Agee, New Orleans, La	.....	12.40	30	22.12	.....	.....	.....	.....
R. B. Blackwood, St. Croix, D. W. I	.....	11.92	30.6	20.28	.....	.....	.....	.....
A. Hugh Bryan, Washington, D. C	2.5	12.40	20	23.77	5.0	13.40	20	23.04
G. H. Hardin, New York, N. Y	2.5	12.00	24	22.94	.....	.....	.....	.....
W. D. Horne, Yonkers, N. Y	1.35	12.00	.....	.....	.....	.....	.....	.....
W. L. Howells, New Orleans, La	5.00	12.80	28	23.78	.....	.....	.....	.....
C. I. Lott, Chicago, Ill	7.7	14.52	24	23.66	12.25	[e] 17.00	24	[e] 23.04
S. F. Sherwood, Washington, D. C	2.0	12.60	20	22.76	4.00	12.80	20	22.34
W. G. Taggart, New Orleans, La	.....	11.20	23	21.62	.....	10.80	23	21.31
M. H. Wiley, New York, N. Y	2.5	12.20	24	23.47	.....	.....	.....	.....
Average	.....	12.40	.....	22.71	.....	12.33	.....	22.23
Basic lead nitrate:	*cc.*				*cc.*			
H. P. Agee, New Orleans, La	4	11.60	30	22.16	.....	.....	.....	.....
R. B. Blackwood, St. Croix, D. W. I	2	12.70	31.4	21.08	.....	.....	.....	.....
A. Hugh Bryan, Washington, D. C	5	12.00	20	24.14	10	13.60	20	23.52
G. H. Hardin, New York, N. Y	5	12.00	24	22.94	.....	.....	.....	.....
W. D. Horne, Yonkers, N. Y	3.6	11.30	22.8	22.63	.....	.....	.....	.....
Do	3.6	11.30	21	22.92	.....	.....	.....	.....
W. L. Howells, New Orleans, La	14	13.40	28	24.25	.....	.....	.....	.....
C. I. Lott, Chicago, Ill	30	14.60	24	23.34	.....	.....	.....	.....
S. F. Sherwood, Washington, D. C	6	12.60	20	22.76	12	13.60	20	21.86
F. G. Smith, St. Paul, Minn	10	12.20	25	23.20	20	13.20	25	21.82
W. G. Taggart, New Orleans, La	2	11.20	23	21.62	4	11.20	23	21.95
M. H. Wiley, New York, N. Y	5	12.00	24	23.47	.....	.....	.....	.....
Average	.....	12.24	.....	22.87	.....	12.90	.....	22.29

[a] 24° Brix.
[b] 1.25 specific gravity.
[c] Saturated solution.
[d] Ten per cent solution.
[e] Not included in average.

On the molasses sample, neutral lead acetate gives the lowest reading, while basic lead acetate gives the highest, the variation being 0.69° with the necessary amount of clarifier and 1.26° with an excess. The individual analyses with some of the clarifiers vary considerably, sometimes as much as 3° or 4°. This is due partly to differences in temperature of polarization and partly to the amount of clarifier used. Horne's clarifier, or dry lead subacetate, gives results lower than the wet lead subacetate, but not so low as neutral lead acetate in this case.

Considering the results obtained during the past two years, the referee recommends that dry lead acetate be adopted provisionally as a clarifier in the examination of cane products. If reducing sugars are present, as in cane molasses and sirup, it has an advantage over the solution of lead subacetate, in that by its use after the volume has been made up the polarization is not affected by the volume of the precipitate. Again, while the solution is diluted a little by its use in excess, the precipitation of the reducing sugars compensates partly for this error. Its value when reducing sugars are not present has not yet been determined, but for cane products it is of special value.

In closing, it is of interest to note that at the last meeting of the International Commission for the Unification of Sugar Methods, held in London on May 31, 1909, it was the consensus of opinion, although no vote was taken, that all polarizations should be made at the standard temperature of 20° C.; that basic lead acetate, either dry or in solution, can be used for polarization, but the greatest care must be used not to introduce an excess of this clarifier; that for reducing-sugar determinations, neutral lead acetate only should be used and never basic lead acetate. To all of these points the association agrees, except that heretofore only the solution was used, and now the dry lead subacetate is recommended as a provisional clarifier.

Recommendations.

It is recommended—

(1) That work on clarifying agents be continued another year, with special reference to the composition of basic lead acetate.[a]

(2) That moisture methods be studied further.

(3) That for dark colored products whose refractive index is to be determined the diluent should be a saturated sugar solution and never water. For formula to be used with the sugar solution see page 178.

(4) That under the method for the determination of copper contained in the precipitate of cuprous oxid,[b] section 6, "Direct weighing of cuprous oxid" be limited by the following statement: "This method should be used only in determinations of reducing sugars in pure solutions. In all other products the copper of the cuprous oxid should be determined by some such method as Low's, Bulletin 107, Revised, page 24, as the cuprous oxid is very apt to be contaminated with organic matter as well as with mineral ash."

(5) That Horne's dry lead subacetate be adopted provisionally as a clarifying agent for polarizing cane and sorghum products in sugar analysis.

DRY LEAD DEFECATION IN RAW SUGAR ANALYSIS.

By W. D. Horne.

To bring this question before the Association of Official Agricultural Chemists, a brief résumé of the history of the subject is almost necessary. At the meet-

[a] Browne, U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 223.

[b] U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, pp. 51–53.

ing of the International Commission for Uniform Methods of Sugar Analysis at Berlin in 1903, Wiechmann called attention to the need of a method obviating the error in polarizing sugar solutions due to the volume occupied by the lead precipitate, which causes too great a concentration and so too high a reading of the polariscope. In December of the same year the writer proposed the use of anhydrous lead subacetate.[a] Later Watts and Tempany corroborated these results and advocated the adoption of the proposed method by the commission. H. and L. Pellet raised objections, however, to the method in 1905,[b] which were answered in an article published in December of the same year in the same journal. But Pellet still claimed[c] that the precipitate absorbed and occluded enough sucrose during its formation to offset the rise in polarization due to concentration. This article was answered in a paper before the Sugar Section of the Sixth International Congress of Applied Chemistry at Rome in 1906 (published in the transactions of that congress, page 577), in which for the second time I showed experimentally that the precipitate does not occlude sucrose.

At the meeting of the International Commission at Berne in August, 1906, Wiechmann presented and recommended this method, but H. Pellet opposed it, quoting experiments made by himself and his son, in which they obtained nearly the same polarizations by the ordinary method and by washing the precipitate on the filter free from sugar and polarizing in a correspondingly long tube the dilute filtrate. From this they argue that the precipitate absorbs enough sugar to compensate for the influence of its volume and therefore the ordinary polarization must be correct.

The commission thereupon ordered the reports printed and the matter laid over for further study. Noel Deerr[d] pointed out the explanation of Pellet's results, showing that the dilution of the solution raised the specific rotatory power of the sucrose enough to make the dilute solutions polarize in the long tube as much as the original solution, concentrated somewhat by the presence of the precipitate.

By a new method I was able to show very conclusively[e] that the precipitate does not absorb any sugar. In this investigation the normal weight of a Cuban molasses sugar was precipitated with subacetate of lead solution and after filtering and draining the ratio of sugar to water was determined very accurately first in the filtrate and then in the mass of wet precipitate on the filter, and found to be 29.03 per cent in the former and 28.69 per cent in the latter, showing that there was no enrichment in sugar in that portion of the solution adherent to the precipitate.

Had sufficient sugar been absorbed by the precipitate to offset the error in polarization due to its volume of 0.50 cc the ratio of sugar to water would have been 28.55 per cent in the filtrate and 30.98 per cent in the precipitate portion, or over seven times as great a difference as was found, and in the opposite direction. This absolutely disproves Pellet's assumption of absorption of sugar by the precipitate.

Answering his further contention that dry lead subacetate dilutes the solution (0.37 cc per gram added) and so accounts for the difference between polarizations by the two methods, experiment has shown that when added to pure water, and so completely dissolved, 1.0 gram of the salt only increases the

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[a] J. Amer. Chem. Soc., 1904, **26**: 186.
[b] Bul. Assoc. Chem. Dist., 1905, **23**: 285–291.
[c] Int. Sug. J., 1906, **8**: 17.
[d] Int. Sug. J., 1907, **9**: 13.
[e] J. Amer. Chem. Soc., 1907, **29**: 926.

volume of the solution 0.22 cc, and determinations of lead as chromate in filtrates from muscovado and molasses sugars showed that the quantity of lead was so slight as to account for only 0.044 and 0.042 cc, respectively, amounts too small to affect appreciably the polariscopic reading, even in these samples, representing the lowest grades. In high-grade sugars, which constitute by far the greater portion of the world's supply, the dilution is correspondingly small and becomes quite insignificant.

Thus these authors (H. and L. Pellet) are in the position of having explained away twice as much difference as ever exists, and that by indirect methods, while the most direct methods thoroughly demonstrate the fallacies of their claims and the superiority of the dry defecation over the process heretofore in use.

This reply has been considered conclusive by all recent writers on the subject. Watts and Tempany [a] after an elaborate investigation of the subject say: "Clarification by means of dry anhydrous basic lead acetate involves no appreciable error;" and again, "it appears unnecessary to search for more complicated methods of clarification, for the use of solid anhydrous lead subacetate gives results which are well within the limits of accuracy of ordinary methods of analysis. Noel Deerr [b] says: "The article by Doctor Horne would seem to conclusively prove that the lead precipitate does not entrain sugar."

Prinsen Geerligs [c] admits that the error due to volume of precipitate is quite large when in speaking of the clarification of sugar solutions containing invert sugar by means of subacetate of lead he says, "The increase in polarization is therefore only partly due to removal of levorotating substance, being due also to the huge quantity of insoluble precipitate arising in the heart of the solution."

A. Hugh Bryan,[d] chief of the sugar laboratory, Bureau of Chemistry, at Washington, in his latest report on sugar analysis methods, reports getting a polarization of 89.0° with alumina cream, 89.50° with lead subacetate solution, and 89.05° with dry lead subacetate, which thus compares almost exactly with what he adopted as the true polarization, while the ordinary defecation gave a result 0.5° too high.

At the recent London Congress of Applied Chemistry dry lead defecation was discussed at length in papers by Mr. Bryan and by the writer before the International Commission for Uniform Methods of Sugar Analysis and was admitted by the commission on an equality with the use of the solution. Doctor Herzfeld, president of the commission, said that it is now optional for any association of chemists to adopt it as the official method.

COMPARISON OF METHODS FOR SUCROSE IN SUGAR-HOUSE CONTROL.

By H. P. Agee.

There have been suggested and are now in use several modifications of the official methods for sucrose which are particularly applicable to the analyses of cane or beet juices and the other unconcentrated products in sugar-house control work where rapidity of manipulation is required.

In the accompanying table there is given a comparison of results obtained by four analysts using the official method and three modifications thereof. The

[a] J. Soc. Chem. Ind., **27**: 53.
[b] Int. Sug. J., 1907, **9**: 235.
[c] Ibid., 1908, **10**: 435.
[d] Ibid., **10**: 604.

deviation consists in the case of the Spencer pipette method in the use of a special pipette so graduated that when filled to the mark corresponding to the degree Brix of the solution 52.096 grams (twice normal weight) will be delivered, and this quantity may be clarified, diluted to 100 cc, and treated as usual. This saves time as compared with the use of the balance for weighing the prescribed normal weight or multiple thereof. In the Schmitz method a 100 to 110 cc flask is filled to the 100 cc graduation with juice or the solution to be analyzed, the lead solution for the clarification is added, and the dilution is completed to the 110 cc graduation. From the polarization of this solution the sucrose content may readily be obtained by reference tables.

The Horne dry-lead method, which eliminates the necessity for either weighing or measuring when applied to juices or dilute products, consists merely of the addition of the clarification agent in the form of a specially prepared dry subacetate of lead with subsequent filtration and polarization. The polarization for the normal concentration is obtained by reference to tables. An additional advantage in the polarization of the material in undiluted form over the normal concentration is that any error in reading is divided approximately by four.

The comparative work on these modifications by four analysts at the sugar experiment station, in August, 1908, is presented in tabular form. The work was done on an immature cane juice, which was the only natural product available at that season of the year, and whereas the sucrose is low, the solids not sugar being proportionally higher than in mature cane juice, the material may be considered a fair one for the test.

Comparative sucrose determination on immature cane juice by the official method and three modifications.

Analyst.	Official method.	Spencer pipette method.	Schmitz method.	Horne's dry-lead method.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
B. F. Hochenedel	4.35	4.35	4.31	4.27
A. B. Joffrion	4.35	4.32	4.30	4.27
P. H. Doherty	4.30	4.30	4.30	4.27
R. E. Graham	4.25	4.40	4.21	4.26
Average	4.31	4.34	4.28	4.27

By inspection of the figures it can be seen that the results without exception are in close conformity. It is suggested that the referee for the coming year make a further study in comparing these widely used methods with the view of having them adopted under "Methods for the analysis of sugar-house products."

REPORT OF COMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By F. W. Woll, *Chairman.*

Dairy Products.

It is recommended—

(1) That the methods of analysis of condensed milk presented to the association at its last meeting and referred to the referee for 1909 be adopted as official. These methods are given in the Proceedings of the association for 1908, Bulletin 122, page 158, and Circular 43, page 8, of the Bureau of Chem-

istry, and are for—(1) Preparation of samples, (2) Total solids, (3) Ash, and (4) Lactose.

Adopted.

(2) That the method for inverting sucrose in the sweetened product with citric acid, using 3 per cent of acid and boiling for forty minutes be again tested.

Adopted.

(3) That methods for determining fat in both sweetened and unsweetened milk be studied, giving special attention to those modifications of the Babcock and extraction methods recently brought out in Bulletin 134 of the Indiana Agricultural Experiment Station.

Adopted.

(4) That the standard for Babcock glassware, proposed by E. B. Holland and referred by vote of the association to the referee for 1909 be adopted as the official standard.

(This standard is printed on page 189 of the Proceedings of the Twenty-fifth Annual Convention of the Association, Bulletin 122, also on page 9 of Circular 43, of the Bureau of Chemistry.)

Adopted.

Foods and Feeding Stuffs.

It is recommended—

(1) That the referee continue the study of the determination of acidity in cattle feeds.

Adopted.

Medicinal Plants and Drugs.

It is recommended—

(1) That the provisional methods for the determination of alkaloids designated as "(a) Total Extraction Method" and "(b) Aliquot Method" (Bulletin 107, Revised, p. 258), with the modifications introduced in 1908 (Bulletin 122, p. 130), be replaced by the modifications suggested by the referee. (See page 192, also Cir. 52, page 14, for full statement of method.)

This recommendation was referred to the association for action in 1910.

(2) That the method for the separation of acetanilid, caffein, and sodium bicarbonate substantially as formulated for work during the past year be made provisional.

This recommendation was referred to the association for action in 1910.

(3) That the method for the separation of acetphenetidin, caffein, and sodium bicarbonate be subjected to additional study.

Adopted.

(4) That the work of the study of microscopical and macroscopical methods and of the micro-chemical study of drugs be continued along present lines.

Adopted.

Sugar.

It is recommended—

(1) That work on clarifying agents be continued another year, with special reference to the composition of basic lead acetate.

Adopted.

(2) That moisture methods be studied further.

Adopted.

(3) That for dark-colored products whose refractive index is to be determined, the diluent should be a saturated sugar solution and never water. (For formula to be used with the sugar solution, see page 178.)

This recommendation was referred to the referee, to be brought before the association for action in 1910.

(4) That under the method for the determination of copper contained in the precipitate of cuprous oxid (pp. 51–53, Bulletin 107, Revised), section 6, "Direct weighing of cuprous oxid," page 53, be limited by the following insertion: "This method should be used only in determinations of reducing sugars in pure solutions. In all other products the copper of the cuprous oxid should be determined by some such method as Low's (Bulletin 107, Revised, p. 241), since the cuprous oxid is very apt to be contaminated with organic matter, as well as other mineral ash."

Adopted.

(5) That Horne's dry lead subacetate be adopted provisionally as a clarifying agent in sugar analysis for the polarization of cane and sorghum products.

Adopted.

REPORT OF COMMITTEE ON RESOLUTIONS.

By F. W. Woll, *Chairman.*

(1) *Resolved*, That we express to our president, Doctor Bigelow, our sincere appreciation of the able and courteous manner in which he has presided over the deliberations of this convention.

(2) *Resolved*, That we express our heartfelt appreciation and thanks to the citizens of Denver and to the committee in charge, to the Chamber of Commerce and the Western Association of Technical Chemists and Metallurgists for the entertainments which were provided for our association and which materially contributed to the enjoyment of our members during their stay in this beautiful city.

The report of the committee was approved.

REPORT ON TANNIN.

F. P. Veitch, *Referee.*

Though the methods for the analysis of tanning materials are not entirely satisfactory, they yield in the hands of careful analysts fairly concordant results, and further study does not promise to lead to their material improvement. For these reasons the referee has turned his attention to the allied subject of methods for the analysis of leather, which is of growing importance, not only because of adulteration and injury caused by the several processes of tanning, but also because of the increasing employment of new tanning materials and their effect on the wearing quality of leather made therewith. Large quantities of leather are employed in the agricultural industries, and it is altogether appropriate that methods for the examination of these articles should be at the service of the agricultural chemist.

Two samples of oak-tanned leather were sent out for examination, No. 1 being the leather as received and No. 2 the same adulterated with 4 per cent of glucose and 8 per cent of hydrated magnesium sulphate. It was requested that the samples be analyzed by the methods of the American Chemists' Association,[a] with certain modifications to be described later. Unfortunately only the following results, obtained by two workers in the Leather and Paper Laboratory of the Bureau of Chemistry, have been reported:

[a] J. Amer. Leather Chem. Assn., 1909, **4**: 124.

Analytical Results.

Results of the analysis of leather.

Determinations.	Sample No. 1.		Sample No. 2.	
	Rogers.	Stewart.	Rogers.	Stewart.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Moisture (fifteen hours at 98° C.)	7.4	6.8	9.7	9.6
	7.4	7.0	9.6	9.6
Petroleum ether extract	5.7	.....	4.3	.....
	5.7	.....	4.3	.....
Ash	.3	.4	1.9	1.9
	.4	.3	1.9	1.9
Magnesium sulphate ($MgSO_4+7H_2O$)	0	0	4.0	4.0
	0	0	4.0	3.9
Water-soluble at 60° C.	16.5	16.1	26.4	29.4 \ 14
	16.7	15.6	27.6	26.0 / hrs.
Water-soluble at 30° C.	12.9	.....	24.7	.....
	12.7	.....	21.8	.....
Soluble nontannin at 60° C.	3.2	3.2	15.1	15.1 \ 14
	3.2	2.8	15.7	15.7 / hrs.
Soluble nontannin at 30° C.	2.9	.....	15.3	.....
	2.7	.....	15.0	.....
Soluble tannin at 60° C.	13.2	12.9	11.3	14.3 \ 14
	13.5	12.8	11.9	10.6 / hrs.
Soluble tannin at 30° C.	10.0	.....	9.4	.....
	10.0	.....	6.8	.....
Soluble glucose at 60° C.	.8	.7	8.2	8.1 \ 14
	.7	.8	8.4	8.3 / hrs.
Soluble glucose at 30° C.	.8	.....	8.5	.....
	.8	.....	8.4	.....
Nitrogen hide substance	37.3	37.3	31.1	31.1
Combined tannin (by difference)	32.7	.....	28.1	.....

The following results imply that fifteen hours' drying is required at 98° C. to secure constant weight on sole leather. Sample No. 2, containing hydrated magnesium sulphate, did not take longer to dry than sample No. 1.

Moisture determinations with varying periods of drying at 98° C. (Rogers).

Periods of drying.	Sample No. 1.	Sample No. 2.	Periods of drying.	Sample No. 1.	Sample No. 2.
	Per cent.	*Per cent.*		*Per cent.*	*Per cent.*
5 hours	7.0	9.2	20 hours	7.4	9.7
	6.9	9.2		7.4	9.7
10 hours	7.0	9.3	25 hours	7.4	9.7
	6.9	9.1		7.4	9.7
15 hours	7.4	9.7			
	7.4	9.6			

Mr. Stewart obtained the following results on glucose in a comparison of basic and normal lead acetate as a clarifying agent. The higher results where the normal acetate was employed are quite striking.

Comparison of glucose determinations in leathers using two clarifying agents.

Sample number.	Normal lead acetate.	Basic lead acetate.	Sample number.	Normal lead acetate.	Basic lead acetate.
	Per cent.	*Per cent.*		*Per cent.*	*Per cent.*
Sample No. 2 (official)	8.1	6.4	2077	9.4	7.8
	8.2	6.2	2114	8.3	6.5
1400	13.7	11.7	2116	9.2	6.9
1405	.14	.16			

Discussion of Results.

No definite conclusions can be drawn from so few results. It may be said, however, that the results on moisture, ash, magnesium sulphate, and glucose are as consistent as can be expected. It is not probable that moisture can be determined so closely in all kinds of leather as has been done in these, nor is it likely that different workers will get such closely agreeing results on ether-soluble material, as it is well known that both of these constituents are very difficult to determine.

The results further indicate that glucose, when present, is quite easily extracted, as much being obtained in the extract at 30° as at 60° C.

The results on total water-soluble material and on tannins and nontannins show wide differences, which the referee believes are to be attributed to the difficulty of extracting so large a quantity of material, and to differences in frequency of siphoning. Mr. Rogers observed that where duplicate extractions did not proceed at the same rate the duplicates did not agree, and the slower extraction gave the lower result. It is essential, therefore (particularly in leather analysis where the constituent determined is never completely extracted), that a definite number of siphonings be made in all cases. It is interesting to note that the differences in water-soluble data are due almost entirely to the tannin, the soluble nontannins being quite constant at different extraction temperatures, and by different analysts. It is not possible to extract the uncombined tannin without removing a small quantity of combined tannin, and these results show how difficult it is to determine the proper stopping point. The procedure, in any case, must be arbitrary, but the referee's experiments indicate that the procedure outlined most accurately distinguishes between combined and uncombined tannin.

Recommendations.

The referee would recommend that work on leather be continued with reference to securing more concordant results on the water-soluble and on the petroleum extract, and especially for the purpose of developing tests that should be indicative of the wearing quality of leather in service.

It is also recommended that as a guide for future work and for use until some definite action can be taken the following methods for the analysis of leather be published:

Proposed methods for leather analysis.

Preparation of sample.—Reduce the sample to as firm a state of division as practicable, being careful to avoid heating it.

Moisture.—Dry 10 grams of leather for fifteen hours at a temperature between 98° C. and 100° C. Cool in desiccator and weigh.

Ether extract.—Extract 5 to 10 grams of air-dried leather with petroleum ether boiling between 60° C. and 80° C., in a Soxhlet apparatus until free from grease. Evaporate the ether and dry to approximately constant weight, cool in desiccator and weigh. (If preferred, extract 15 grams of leather as described above, in which case, the extracted leather, when freed of solvent, may be used for the determination of water-soluble material.)

Ash.—Incinerate 10 grams of leather in a tared dish at a dull red heat until carbon is consumed. If it is difficult to burn off all the carbon, treat the ash with hot water, filter through an ashless filter, ignite the filter and the residue, add the filtrate, evaporate to dryness, and ignite again; cool in desiccator and weigh.

Water-soluble material.—Treat 15 grams of leather in a soxhlet or extractor that will allow the continuous percolation of liquid for fourteen hours, at the same time keeping the volume of the percolated liquid within one liter, the extraction to be made at 50° C. The first 200 cc should be collected in an outside flask without boiling while the subsequent solution in the receiving flask is changed and combined with the first extract as concentration demands. Determine total solids, nontannin, and glucose in this extract.

Glucose.—Treat 200 cc of the water extract with 25 cc of neutral lead acetate. Shake well, let stand for one hour and filter and treat with a small excess of potassium oxalate. Filter, and to 150 cc of the filtrate add 5 cc of hydrochloric acid and boil down to 175 to 190 cc; then boil for two hours with a reflux condenser; cool, neutralize, filter if necessary, and make up 200 cc; determine glucose by Allihn's modification of Fehling's method, Bulletin 107, Revised, page 49.

Nitrogen.—Determine by the Kjeldahl or Gunning method, Bulletin 107, Revised, pages 5 to 7.

Hide substance.—Multiply the percentage of nitrogen by 5.62, and the result will be the percentage of hide substance present.

REPORT ON THE ASSAYING OF ALKALOIDAL DRUGS.

By C. E. Parker, *for Referee on Drugs.*

General Discussion.

The work on the samples of cinchona (red and yellow) and colchicum corm and seed which were distributed for assay in 1908 was not completed in season to report to the last convention and it is therefore reported at this time. The methods appearing in Bulletin 107, Revised, pages 259 and 260, were directed to be used for these samples, with the single modification that in Method II, for cinchona, collaborators were instructed to let the drug stand overnight in extracting. This year samples of aconite root, belladonna leaves and root, and a few samples of cinchona have been distributed with instructions to about 20 collaborators, but not enough results have been received on these samples to warrant making a report.

Pharmacopœial requirements as to fineness of powdered drugs are not closely followed in the drug trade, and there is sometimes difficulty in purchasing drugs of standard fineness for assaying. A number of the drugs sent out in 1908 for cooperative work deviated unavoidably from the standard in this respect, and due caution must be observed in drawing conclusions from the results.

In certain cases the U. S. P. methods sanction the use of a mechanical agitator in extracting the drug, but more often "shaking at frequent intervals," etc., is directed. This may be differently interpreted with resulting variations, and continuous agitation by mechanism is recommended as an improvement.

Collaborators sometimes had difficulty in decanting a 100 cc aliquot. This is believed to have been due to the coarseness of the powders and an expedient for obtaining a filtered aliquot without much evaporation will be suggested, which it is believed will obviate this trouble.

A possible source of error has been located in the deposition of alkaloid from the evaporation of the volatile solvent on the outside of the tips of separators, funnels, and percolators, edges of filter papers, etc., and a recommendation will be made respecting the recovery of this alkaloid.

In dissolving alkaloidal residues in tenth-normal acid for titration there is a liability to error in measuring the few cubic centimeters of acid usually directed. Solution in an equivalent amount of fiftieth-normal acid is often tedious. It is proposed to try more thoroughly the expedient of dissolving in alcohol, adding water, indicator, slight excess of fiftieth-normal acid, and titrating back with fiftieth-normal alkali, comparing the result with that of a blank run in parallel. It is thought that the use of alcohol will save time and prevent the precipitation of alkaloid near the neutral point, which tends to obscure the end reaction.

It is believed that losses may be prevented by checking the completeness of exhaustion in percolation and shaking out by means of alkaloidal tests, also in washing acid alkaloidal solutions with ether, etc., by washing the latter

with water before discarding it, and adding the water to the acid. Provision will be made for this in modifications of the methods. (See Cir. 52, p. 14.)

The suggestion is made that in preparing ether-chloroform ammonia mixtures of the Prollius type, a slight excess of the mixture should be made and allowed to cool and settle before measuring the amount for extraction of the drug, the aqueous layer being rejected.

Analytical Results and Their Discussion.

The following table contains the results reported on the 1908 samples of cinchona and colchicum:

Cooperative results on determination of alkaloids.

Collaborators.	Cinchona, Red.				Cinchona, Yellow.				Colchicum corm.		Colchicum seed.	
	Method I (U. S. P.).		Method II. Total extraction.		Method I (U. S. P.).		Method II. Total extraction.					
	Total.	Ether soluble.	Dried at 70°.	Dried at 110°.	Total.	Ether soluble.	Dried at 70°.	Dried at 110°.	Method I (U. S. P.).	Method II.	Method I (U. S. P.).	Method II.
	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
Benton, Hall & Co	7.300	.....	[a]7.595	.....	6.390	5.840	[a]6.730	.....	[ab].310	.273	[b].580	.350
	7.270	.....	[a]7.385	.....	6.320	5.860	[a]6.585	.....	[ab].332	.293	[b].620	.365
Darling, J. F	7.066	6.660	7.340	7.111	6.350	5.598	6.628	6.621	.360	.324	.502	.367
	7.068	6.488	7.386	7.156	6.392	5.754	.....	.....	.414	.314	.....	.393
Fuller, H. C	.....	.....	.....	.....	6.790	[c]6.430	[d]6.400	.....	[d].370	.340	[d].740	.790
	.....	.....	.....	.....	6.880	[c]6.770	[d]6.400	.....	[d].380	.360	[d].760	.770
Heyl, F. W	6.850	6.420	.....	[e]7.150	6.250	5.730	.....	6.490	.....	.....	.....	.....
	7.020	6.520	.....	[e]7.080	6.280	5.740	.....	6.480	.....	.....	.....	.....
	7.110	6.570	.....	.....	.....	.....	.....	6.500	.....	.....	.....	.....
	7.070	6.620	.....	.....	.....	.....	.....	6.520	.....	.....	.....	.....
Lilly & Co., E	[f]6.680	[g]6.510	7.780	.....	[f]6.408	[g]6.260	7.370	.....	[h].436	[i].484	[k].530	[m].540
	[f]6.730	[g]6.500	7.750	.....	[f]6.440	[g]6.230	7.340	.....	[i].438	[j].487	[l].500	[n].552
Lyons, A. B	7.230	6.680	.....	7.350	6.350	5.410	.....	6.540	[o].440	[q].330	[r].570	[t].510
	7.380	6.780	.....	7.420	6.590	5.880	.....	6.640	[p].414	.....	[s].624	[u].487
Nelson, B. E	6.415	.....	7.170	.....	4.798	.....	5.394	.....	[v1].550	[v1].505	.576	.470
	6.475	.....	6.982	.....	4.795	.....	5.479	.....	[v1].710	[v1].585	.600	.467
Parke, Davis & Co	7.274	6.884	7.522	.....	6.588	6.180	6.780	.....	.340	.312	.570	.390
	7.384	6.850	7.480	.....	6.526	6.060	6.815	.....	.356	.314	.830	.398
Parker, C. E	[d]6.920	[g]6.790	[d]7.739	7.564	[d]6.366	[g]6.070	[d]6.729	6.194	[d].408	[w].240	[d].600	[x].335
	[d]7.210	[g]6.730	[d]7.739	7.572	[d]6.430	[g]6.210	[d]7.053	6.437	[d].414	[w].238	[d].568	[x].329
Ruddiman, E. A	[y]6.866	4.062	6.868	.....	[y]5.806	[v2].704	6.178	.....	.334	.304	[z].356	.320
	[y]6.832	3.992	6.733	.....	[y]5.882	[v2].678	6.193	.....	.332	.313	[z].320	.338
Sayre, L. E. (Ziefle, A.)	.....	.....	5.665	.....	5.514	5.180	4.711	.....	.398	.238	.918	.431
	.....	.....	5.686	.....	5.586	4.990	4.750	.....	.384	.229	.928	.450
Seil, H. A	7.130	6.860	7.392	7.171	6.290	6.060	7.097	6.773	.388	.278	.476	.354
	7.010	6.710	7.334	7.124	6.330	5.960	7.191	6.742	.370	.271	.488	.382
Smith, Kline & French Co.	6.984	6.648	7.650	.....	6.648	5.606	7.300	.....	.366	.219	.618	.359
	6.982	6.696	7.660	.....	6.646	5.616	7.301	.....	.366	.220	.620	.359
Average	7.011	6.398	7.243	7.272	6.217	5.883	6.496	6.540	.380	.304	.604	.438
Per cent within 10 per cent of average	100	90	90	100	81	86	57	100	64	43	52	21
Per cent within 15 per cent of average	100	90	90	100	92	91	81	100	86	57	52	38

[a] The volatile solvent was filtered through a cotton plug in stem of separator.
[b] Shook frequently for four hours, let stand overnight, then shook frequently for eight hours.
[c] Temperature may have been above 20° C.
[d] Used mechanical agitator.
[e] Let macerate three days before percolation.
[f] In the final shaking out more volatile solvent was used.
[g] Contents of separator kept at 15° C. in shaking out.
[h] Soluble in water 0.398 per cent.
[i] Soluble in water 0.394 per cent.
[j] Soluble in water 0.355 per cent.
[k] Soluble in water 0.44 per cent.
[l] Soluble in water 0.456 per cent.
[m] Soluble in water 0.453 per cent.
[n] Soluble in water 0.459 per cent.
[o] Titrated with Mayer's solution 0.40 per cent.
[p] Titrated with Mayer's solution 0.43 per cent.
[q] Titrated with Mayer's solution 0.31 per cent.
[r] Titrated with Mayer's solution 0.50 per cent.
[s] Titrated with Mayer's solution 0.53 per cent.
[t] Titrated with Mayer's solution 0.49 per cent.
[u] Titrated with Mayer's solution 0.48 per cent.
[v] Not included in average.
[w] Used 250 cc alcohol to extract.
[x] Used 225 cc alcohol to extract.
[y] Digested with 150 cc instead of 300 cc of solvent mixture.
[z] The alkaloid was purified as directed for colchicum corm.

CINCHONA, RED.

This sample was delivered as No. 80 powder, and passed through the several sieves in the following proportions:

No.	Grams.
80	60
60	5
50	34
Total	99

Though the powder was not of standard fineness the results by both methods show a gratifying degree of uniformity, and more than has been attained with cinchona assays in previous years, when the ether soluble results especially were variable. Most of the results by Method I, however, are no doubt too low. It is an interesting circumstance that by Method II the average results when drying at 110° C. are higher than those obtained by drying at 70°. Evidently those workers who dried the residue at 110° C. on the whole came nearer to exhausting the drug. Unquestionably the cinchona alkaloids should be dried at a higher temperature than 70° and probably higher than 110°; according to the U. S. Pharmacopœia (page 373, line 26), a suitable temperature would be 125°.

By Method I the amounts of drug and solvent mixture prescribed are objectionably large. Mr. Parker obtained by shaking out with three additional portions of acid 0.03 to 0.04 per cent more alkaloid. Another duplicate by this method yielded 7.36 and 7.32 per cent of total alkaloid.

By Method II the exhaustion of the drug was tedious. After percolating with 400 cc of menstruum, when the test with Mayer's solution gave practically no reaction, Mr. Parker obtained from the marc about 0.02 per cent more alkaloid. There was a somewhat troublesome emulsification in shaking out.

CINCHONA, YELLOW.

This sample was delivered as No. 80 powder, and passed through the respective sieves in the following proportions:

No.	Grams.
80	80.5
60	14.5
50	4.5
40	.5
Total	100

Though this powder was nearer the standard fineness than the cinchona, red, the results varied more, those by Method II drying at 70° C. being decidedly poor, while those obtained by drying at 110° C. are good. It will be noted that in this case also the averages by drying at 110° C. are higher than those by drying at 70° C., indicating more complete extraction in certain cases. Many results by Method I are too low, the drug evidently containing at least 6.5 per cent of total alkaloid dried at 110°. The amounts of drug and solvent mixture required by Method I are too large, and the amounts of acid and ether-chloroform directed for shaking out are hardly adequate. Mr. Parker

shook out with four more portions of acid, obtaining 0.15 and 0.14 per cent of alkaloid. In shaking out ether-soluble alkaloid the ether and acid solution were brought to 10° C. by setting the separator in cold water, adding ammonia water, shaking the separator two minutes, and placing in a water bath at 15° C. for ten minutes. The aqueous layer was drawn off by the tap, the ether decanted through the mouth of the separator, and the latter rinsed out with cooled ether. The total alkaloid always is slightly contaminated and discolored and a purification may be practicable.

By Method II considerable emulsification was encountered in shaking out. The amount of drug might be reduced with advantage, as the exhaustion is tedious and requires much menstruum. More acid and ether-chloroform are advisable in the shaking out. Mr. Parker extracted with three more portions of ether-chloroform 0.413 and 0.146 per cent of alkaloid. Mr. Fuller determined ether-soluble alkaloids at 15° C. in the alkaloidal residue obtaining 5.3 per cent; he observed that the marc was not quite exhausted. Mr. Lyons has obtained good results with Fromme's aliquot method, tried as Method II in 1906, and urges that it be given further investigation.

The utility of systematic comparison of total extraction with aliquot methods is indicated by the present cinchona results, which show rather conclusively that the aliquot results are too low.

COLCHICUM CORM.

This sample was delivered as No. 60 powder and passed through the respective sieves in the following proportions:

No.	Grams.
60	23
50	15
40	24
20	37
Total	99

The powder therefore deviated much from standard fineness, which may be partly responsible for the excessive variations in results by both methods. The average obtained by Method I is higher than that by Method II, and while no conclusion is warranted, Method I may give too high results for the following reasons: If any suspended ammonia water is included in the 100 cc of solvent mixture with which the drug is digested, the 50 cc of aliquot filtered off would represent more than half, and evaporation during filtration, if not prevented, would increase the error. Notwithstanding the purification of the alkaloidal residue directed in Method I, the final residue was in some cases found to be slightly contaminated as well as that obtained by Method II. The results by Method II are apparently for the most part too low. The method works well, but the evidence seems to indicate that 95 per cent alcohol is not a good menstruum for exhausting this drug. Mr. Lyons suggests that 80 per cent alcohol would be better, and also reports good results by digesting with a dilute solution of lead subacetate and finishing the percolation with warm water. He prefers titration with Mayer's solution[a] to the gravimetric determination of colchicine. Mr. Seil shook out the petroleum ether instead of evaporating with water.

[a] American Druggist, 1909, **54**: 65.

The sample was delivered as No. 60 powder and passed through the respective sieves in the following proportions:

No.	Grams.
60	21
50	16
40	18
20	42
Total	97

This powder also was mostly too coarse, which may have been partially responsible for the very poor results. The average by Method I is again higher than that by Method II, which may be attributable to the causes mentioned under colchicum corm.

In Method I the filtration of the so-called "aqueous solution" is very unsatisfactory. The apparent object is a separation of the oily residue from the aqueous solution, but the oil clogs the paper and more or less passes through. There is no measure directed to prevent its passing on into the alkaloidal residue, which in fact was found to be quite impure whenever tested. Besides remedying this defect, the alkaloid from the seeds should be purified as well as that from the corm. Mr. Fuller found that the oily residue retains alkaloid, and obtained an appreciable amount on shaking out with acid. There is considerable emulsification in shaking out. Most of the results by Method II are evidently too low, owing to the difficulty of exhausting the drug with 94 per cent alcohol. Mr. Fuller practically exhausted the drug in obtaining 0.79 to 0.77 per cent, but did not test the purity of the alkaloid. Mr. Lyons' titrations indicate that the drug contained at least 0.50 per cent colchicin. Doctor Seil shook out the petroleum ether instead of evaporating it with water. There is considerable emulsification in shaking out. Both methods in their present forms are unsatisfactory.

REPORT ON HEADACHE MIXTURES.

By W. O. Emery, *for Referee on Drugs.*

A study of methods for the estimation of the more important constituents of headache mixtures was inaugurated one year ago and began with the examination of a simple powder made up of acetanilid, caffein, and sodium bicarbonate. The results submitted by the collaborators and reported at the Washington meeting were quite gratifying in view of the newness of the method and the short time limit set for the completion of the work. It was deemed advisable by the referee to subject the method to additional study, and this has accordingly been done during the past year. A circular letter, accompanied by the amended methods to be followed, was sent out in December, 1908, to such chemists as had previously reported or expressed a willingness to cooperate. Shortly thereafter two additional headache mixtures were distributed, the one containing acetanilid, caffein, sodium bicarbonate, and sugar; the other acetphenetidin, caffein, and sodium bicarbonate. In this connection it should perhaps be mentioned that all three mixtures submitted to collaborators were compounded of air-dried materials obtained from manufacturers of established reputation.

Methods of Analysis.

The methods submitted for study were as follows:

ANALYSIS OF HEADACHE POWDERS.

Acetanilid mixture, containing caffein, acetanilid, sodium bicarbonate, and sugar.

Caffein.

Weigh out about 0.3 gram of headache powder on a small (5.5 cm) tared filter,[a] wash with successive small portions of chloroform to the amount of 30 to 40 cc, collecting the solvent in a 100 cc erlenmeyer. Distil off chloroform by means of a small flame until only a few cubic centimeters remain. Add 10 cc of dilute sulphuric acid (1 volume concentrated acid to 5 of water) then continue the distillation till all the chloroform has gone over, rotating the flask slightly to avoid overheating. Disconnect from condenser, transfer to a steam or hot water bath, and digest until contents of flask have evaporated to about 4 or 5 cc.[b] This result is attained usually in two to three hours. Cool, then pour and rinse into a separatory funnel, so that the final volume does not greatly exceed 20 cc. Add four times the volume, in this case 80 cc of chloroform, shake for some time vigorously, allow to stand until the chloroform clears perfectly, then pass through a small (5.5 cm) dry filter into a 100 cc erlenmeyer, distil off the solvent till only about 5 cc remain in the flask; then use the distillate for a second extraction, observing the same procedure in shaking, clearing, and filtering as above noted.[c] Distil off most of the chloroform, transfer residue to a small tared 50 cc crystallizing dish or beaker by means of small quantities of chloroform. Allow to evaporate on a vapor bath at a moderate temperature, partially covering the dish with a watch glass toward the end of the operation, in order to avoid possible loss by decrepitation.[d] Cool in desiccator and weigh.

Acetanilid.

The acid solution remaining in the separator and containing the anilin sulphate is run into a 100 cc erlenmeyer and heated a short time on the steam bath to expel traces of chloroform. The filter used in the preceding operation to dry the chloroform solution of caffein is washed once with a little water, the

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[a] The filter may be conveniently tared in the following manner: Fold and fit to short-necked funnel (the latter resting on a glass support) then allow to remain one-half hour in balance case prior to weighing.

[b] During digestion on steam bath the solution of acetanilid, more especially of acetphenetidin when this substance is present, will be greatly expedited if the flask is gently rotated during the first part of the operation. Furthermore, the dissipation of aqueous acetic acid can be greatly accelerated by surrounding the flask with an asbestos mantle or chimney.

[c] Practically the same result is obtained by means of three extractions, using 60 cc of chloroform each time instead of 80 cc as above indicated. In this connection it may not be amiss to emphasize the necessity of exercising extreme care in transferring the dissolved caffein from one vessel to another. Small amounts accumulate at the apex of the funnel, the edge of the filter, and on the rim of the erlenmeyer. These must all be regained through proper manipulation of fresh chloroform. Since cork stoppers yield appreciable amounts of extractive matter to chloroform, all stoppers used in connection with this reagent should be previously treated with boiling chloroform, which is subsequently regained and employed in extraction work.

[d] The evaporating dish containing caffein should be removed from the steam bath just as soon as the odor of chloroform can no longer be detected. If the crystals are not colorless or nearly so, they may be dissolved in about 10 cc of 1 per cent hydrochloric acid, filtered when cold if necessary, then treated with 15 to 20 cc of Wagner's reagent, allowed to stand one-half hour, filtered, and the precipitate washed with a few cubic centimeters of the iodin solution, the precipitate together with filter transferred to separator, decolorized by means of a small crystal of sodium sulphite and the caffein finally extracted with chloroform substantially as carried out at first.

latter running into the separator. Considerable care should be exercised in rinsing out the separator and washing its stopper thoroughly, all rinsings to be added to the sulphuric acid solution. To this solution are added 3 to 5 cc of concentrated hydrochloric acid,[a] then a standard solution of potassium bromid-bromate slowly run into a faint but distinct yellow coloration.[b] While adding this reagent, or after each small addition, the flask should be rotated sufficiently to agglomerate the precipitated tribromanilin and thus sufficiently clear the supernatant liquid. The number of cubic centimeters required multiplied by the value of 1 cc in terms of acetanilid, will give the amount of acetanilid present in the mixture.

Sodium bicarbonate and sugar.

The residue left after first treatment with chloroform, and consisting in the present instance of sodium bicarbonate and sugar of milk, is permitted to stand twenty-four hours in the open, then one-half hour in the balance case and is weighed. The residue may then be ignited, with the usual precautions, with sulphuric acid and the resulting sodium sulphate weighed, or it may be titrated with tenth-normal sulphuric acid, using congo red, preferably, as indicator. The amount of sodium bicarbonate having been determined by either one or both these methods, the quantity of sugar present is then readily obtained by difference.

Acetphenetidin mixture, containing caffein, acetphenetidin, and sodium bicarbonate.

Caffein.

The procedure is precisely the same as that already outlined under acetanilid mixture.

Acetphenetidin.

The acid solution containing the phenetidin sulphate is treated with successive small portions of sodium bicarbonate until an excess of this reagent is observed in the bottom of separator. Then add 50 cc chloroform and 15 to 20 drops of acetic anhydrid, shake for some time vigorously, allowing the chloroform to become perfectly clear, when it is passed through the same filter used for drying the chloroform solution of caffein, into a 100 cc erlenmeyer. After distilling off most of the chloroform over a small flame, the distillate is again used for a second extraction, this procedure being carried out in the same manner as the first. After distilling the chloroform down to a small volume, the residue is very carefully transferred to a 50 cc crystallizing dish by means of small quantities of chloroform. The solvent is then allowed to evaporate on the steam bath, all traces of acetic anhydrid being removed through repeated additions of a few drops of absolute or strong alcohol together with one to two cubic centimeters of chloroform. The acetphenetidin will finally appear as a dry, white crystalline mass, which is cooled in the desiccator and weighed.

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[a] Hydrochloric acid appears to induce a more rapid clearing of the solution during titration than sulphuric acid. The former acid can not, however, be used to advantage in hydrolyzing acetanilid or acetphenetidin for the work in hand, since the corresponding hydrochlorids are not sufficiently insoluble in chloroform to permit of their quantitative separation from caffein.

[b] For the purpose in question the standard solution is prepared by adding bromin in slight excess to a concentrated aqueous solution of caustic potash (50 grams), then diluted to dissolve any separated salts, boiled to expel excess of bromin and finally made up to 1 liter. This solution may be standardized with weighed amounts of pure anilin (Reidel, Zts. physik. Chemie., 1906, **56**: 244), or better with weighed amounts of acetanilid (Seidell, J. Amer. Chem. Soc., 1907, **29**: 1091), adjusting by proper dilution so that 1 cc is exactly equivalent to 1 centigram of acetanilid. For purposes of titration and to insure greater accuracy of results the acetanilid should be recrystallized from hot, very dilute, acetic acid, then thoroughly dried in vacuo over lime. One to two decigram samples are then heated two to three hours on the steam bath with 10 cc dilute sulphuric acid, 10 cc dilute hydrochloric acid added and titrated.

Sodium bicarbonate.

The residue left after first treatment with chloroform is weighed after standing twenty-four hours in the open and one-half hour in the balance case. The weight represents the amount of sodium bicarbonate present in the mixture. Calculate results in parts per 100.

Analytical Results.

The results obtained on the first headache mixtures sent out during August and September, 1908, and reported since the Washington meeting, have been tabulated as follows:

Cooperative percentage results on acetanilid mixture,[a] 1908.

Analyst.	Caffein.	Acetanilid.		Sodium bicarbonate.		Total.
		Gravimetric.	Volumetric.	Gravimetric.	Volumetric.	
L. A. Brown, Kentucky	9.94	.....	65.10	24.94	.....	99.98
	(11.24)	.....	(64.22)	(24.88)	.....	(100.34)
W. O. Emery, Washington, D. C	10.06	.....	64.93	24.93	.....	99.92
	9.97	.....	64.73	24.90	.....	99.60
F. F. Flanders, Washington	(10.46)	.....	(62.72)	(25.76)	(24.46)	(98.29)
	(10.46)	.....	(63.84)	(25.76)	(24.23)	(99.29)
C. D. Howard, New Hampshire	9.88	.....	64.83	25.20	25.08	99.85
	10.00	.....	65.05	25.18	25.10	100.19
	(10.06)	.....	(64.83)	.....	.....	.....
C. C. LeFebvre, Washington, D. C	9.87	.....	64.70	24.76	.....	99.33
	10.00	.....	64.62	25.04	.....	99.66
O. S. Marckworth, Ohio	10.20	.....	64.36	24.73	.....	99.29
E. L. Redfern, Nebraska	9.93	.....	65.05	25.20	.....	100.18
	10.46	.....	65.05	24.93	.....	100.44
	(9.53)	(64.86)	(63.41)	(24.80)	.....	(98.47)
	(10.26)	(65.03)	(63.41)	(24.73)	.....	(99.21)
R. R. Shively, Washington, D. C	10.40	.....	64.73	25.10	.....	100.23
	10.20	.....	64.83	25.00	.....	100.03
H. E. Tiffany, Delaware	9.60	.....	64.96	25.77	.....	100.33
	(9.00)	.....	(64.96)	(24.94)	.....	(98.90)
	10.70	.....	64.96	25.49	.....	101.15
	10.35	.....	65.27	25.11	.....	100.73
	9.85	.....	65.36	25.60	.....	100.81
	9.52	.....	64.32	25.02	.....	98.86
	10.47	.....	64.78	24.92	.....	100.17
	10.50	.....	65.23	25.09	.....	100.82
	10.12	.....	64.87	24.96	.....	99.95
Average	10.16	64.89		25.09		100.08
Maximum	11.24	65.36		25.77		101.15
Minimum	9.00	62.72		24.73		97.74
Difference	2.24	2.64		1.04		3.41
Composition of mixture	10.04	64.99		24.96		.....

[a] Caffein anhydrid 70 parts, acetanilid, 453 parts, and sodium bicarbonate, 174 parts.

In the foregoing, as well as the following two tables, all constituent percentages when incomplete or varying one per cent or more from the true values have not been considered in drawing averages. In cases also where two percentages of a single constituent were reported, the mean has been taken in

computing the total percentage. The results reported on the second and third mixtures sent out in 1909 are as follows:

Cooperative percentage results on acetanilid mixture,[a] 1909.

Analyst.	Caffein.	Acetanilid.	Residue.	Sodium bicarbonate.	Sugar.	Total.
W. O. Emery, Washington, D. C.	9.41	60.15	30.08	23.02	7.06	99.64
	9.43	60.17	30.06	22.92	7.13	99.66
	9.48	60.04	29.97	23.05	6.92	99.49
F. F. Flanders, Washington	(10.06)	(59.36)	(28.80)	(23.80)	(5.00)	(98.22)
	(10.33)	(59.92)	(29.36)	(24.36)	(5.00)	(99.61)
	(11.60)	(59.08)	.....	(24.92)	.....	.....
	(11.93)	(59.08)	.....	(24.92)	.....	.....
L. D. Havenhill, Kansas	(10.00)	(60.18)	(31.43)	(23.78)	(7.65)	(101.61)
	(10.04)	(60.18)	(31.35)	(23.92)	(7.43)	(101.57)
	(9.90)	(60.12)	(31.30)	(23.60)	(7.70)	(101.32)
	9.82	59.67	30.63	24.03	6.61	100.12
C. D. Howard, New Hampshire	9.50	59.32	30.60	23.60	7.00	99.42
	9.36	59.32	30.70	23.54	7.16	99.38
	9.38	60.00	30.80	23.60	7.20	100.18
C. C. LeFebvre, Washington, D. C.	9.63	59.72	30.36	23.32	7.04	99.71
	9.75	59.62	30.52	23.28	6.76	99.89
O. S. Marckworth, Ohio	9.98	59.97	29.93	23.83	6.10	99.88
C. B. Morrison,[b] Connecticut	9.97	60.23	30.53	24.24	6.29	100.73
	9.80	60.23	30.37	24.24	6.13	100.40
E. L. Redfern, Nebraska	(9.80)	(60.68)	(29.86)	(22.13)	(7.73)	(100.34)
	9.80	60.68	29.74	22.41	7.33	100.22
R. R. Shively, Washington, D. C.	9.66	60.17	30.50	23.37	6.80	100.33
	9.70	60.07	30.50	23.23	7.00	100.27
	9.65	59.77	30.40	23.35	7.23	99.82
J. J. Wintler,[c] Washington	10.23	59.27	30.83	24.00	6.83	100.33
	10.23	59.27	30.66	24.00	6.66	100.16
Average	9.67	60.07	30.30	23.43	6.83	100.03
Maximum	10.33	60.68	31.43	24.36	7.73	101.61
Minimum	9.36	59.27	28.80	22.13	5.00	98.22
Difference	0.97	1.41	2.63	2.23	2.73	3.39
Composition of mixture	9.33	60.67	30.00	23.33	6.67	.....

[a] Caffein anhydrid, 28 parts; acetanilid, 182 parts; sodium bicarbonate, 70 parts, and sugar, 20 parts.
[b] Reported by J. P. Street.
[c] Reported by C. W. Johnson.

Cooperative percentage results on acetphenetidin mixture,[a] 1909.

Analyst.	Caffein.	Acetphenetidin.	Sodium bicarbonate.		Total.
			Gravimetric.	Volumetric.	
W. O. Emery, Washington, D. C.	9.92	64.83	25.31	.....	100.06
	10.08	64.56	24.86	.....	99.50
F. F. Flanders, Washington	(11.80)	(62.96)	(25.76)	(24.46)	(99.87)
	(12.56)	(63.50)	(26.04)	(24.40)	(101.28)
L. D. Havenhill, Kansas	10.44	65.40	25.43	25.44	101.28
	(11.90)	(64.10)	(25.34)	(25.40)	(101.37)
	10.46	64.90	24.87	25.17	100.38
C. D. Howard, New Hampshire	(9.04)	(61.00)	(25.16)	(25.10)	(95.17)
	(9.20)	(62.28)	(25.14)	(25.02)	(96.57)
	(9.34)	(63.34)	(25.80)	(25.02)	(98.09)
C. C. LeFebvre, Washington, D. C.	10.19	64.80	24.29	24.26	99.27
	10.10	64.85	24.97	24.90	99.89
O. S. Marckworth, Ohio	(15.12)	(59.72)	(25.13)	.....	(99.97)
C. B. Morrison,[b] Connecticut	10.03	65.10	.....	25.45	100.58
	10.07	64.80	.....	25.45	100.32
E. L. Redfern, Nebraska	(10.73)	(66.60)	(24.73)	.....	(102.06)
	(10.26)	(60.00)	(24.73)	.....	(100.99)
R. R. Shively, Washington, D. C.	10.06	64.81	25.16	.....	100.03
	10.12	64.85	25.09	.....	100.06
	9.94	64.69	25.24	.....	99.87
J. J. Wintler,[c] Washington	10.42	64.56	.....	25.50	100.48
	10.20	64.66	.....	25.66	100.52
Average	10.16	64.83	25.02	25.23	100.17
Maximum	15.12	66.60	26.04	25.66	102.10
Minimum	9.04	59.72	24.29	24.26	96.62
Difference	6.08	6.88	1.75	1.40	5.48
Composition of mixture	10.04	64.87	25.09		.....

[a] Caffein, 28 parts; acetphenetidin, 181 parts; and sodium bicarbonate, 70 parts.
[b] Reported by J. P. Street.
[c] Reported by C. W. Johnson.

Discussion of Results.

The following comments were made by the collaborating chemists:

Mr. Brown commenting on the extraction of caffein and acetanilid states that he prefers to use Gooch crucibles with ignited asbestos for residue (soda).

Mr. Howard experienced considerable difficulty in connection with the estimation of acetphenetidin. For the preliminary extraction with chloroform he prefers a tared platinum gooch, placing same in the usual Gooch funnel (without rubber connection) for extraction. By gently waving the gooch with soda residue over a radiator (or other suitable source of heat) a practically constant weight is attained.

Mr. Johnson suggests that a more satisfactory end reaction could be obtained by the use of a more dilute solution of sodium bromid-bromate.

Mr. Havenhill does not favor the use of tared filters, having, as he says, discarded them years ago for counterpoised filters. While these latter require more time, he finds them uniformly satisfactory.

Mr. Flanders employed methyl orange as indicator instead of congo red.

Mr. Tiffany found it expedient to thoroughly boil out all corks used with chloroform prior to extraction with this medium, otherwise he invariably obtained too high results for caffein.

Commenting on the work in general it would appear that very satisfactory results can be obtained by following the directions substantially as submitted to the collaborators. The bureau workers have had occasion to use these methods as a basis in investigating several hundred headache mixtures of widely varying composition. Slight variations like those already suggested,

as well as others, can of course be introduced without materially changing the effectiveness of the method. Some will prefer tared filters, while others may incline toward Gooch crucibles or counterpoised filters. Mr. Shively after using tared filters, for a time resorted to Gooch crucibles, but finally returned to the original procedure in the belief that it was subject to less error. In titrating anilin sulphate he makes use of a solution of sodium bromid-bromate, 1 cc of which is equivalent to 5 mg of acetanilid.

Many of the collaborators are apparently experiencing no little difficulty with the separation of caffein and acetphenetidin and in truth close attention to detail is necessary for the proper hydrolysis of this latter substance. Its properties are such that it not only goes into the acid solution very slowly but also has a tendency to collect in small particles on the glass above the liquid. This difficulty (and in some cases the cause of high caffein and low acetphenetidin) may be obviated by frequently rotating the flask during the first hour of heating, so that no particle of acetphenetidin is left clinging to the glass. Another source of error may possibly be located in the too early addition of acetic anhydrid. This should be effected only after complete neutralization with sodium bicarbonate.

Recommendations.

In view of the results thus far recorded the referee feels justified in recommending to the association that the method for the separation of caffein and acetanilid substantially as last formulated, be made provisional; and further that the method for the estimation of acetphenetidin receive additional study. It is also recommended that additional mixtures be tested with such other methods as may be found desirable.

The convention adjourned.

OFFICERS, REFEREES, AND COMMITTEES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1909–10.

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W. A. Withers, Raleigh, N. C.

Vice-President.

F. W. Woll, Madison, Wis.

Secretary.

H. W. Wiley, Washington, D. C.

Additional members of the Executive Committee

J. M. Bartlett, Orono, Me.
J. T. Willard, Manhattan, Kans.

Referees.

Phosphoric acid: W. F. Hand, Agricultural College, Miss.
Nitrogen:
Determination of nitrogen: C. H. Jones, Burlington, Vt.
Separation of nitrogenous bodies (meat proteids): C. R. Moulton, Columbia, Mo.
Potash: E. L. Baker, Geneva, N. Y.
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Sugar: H. P. Agee, Audubon Park, New Orleans, La.
Tannin: J. S. Rogers, Washington, D. C.
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Inorganic plant constituents: O. M. Shedd, Lexington, Ky.
Medicinal plants and drugs: L. F. Kebler, Washington. D. C.
Water: J. K. Haywood, Washington, D. C.

Associate referees.

Phosphoric acid: H. D. Haskins, Amherst, Mass.
Nitrogen:
Determination of nitrogen: J. W. Kellogg, Harrisburg, Pa.
Separation of nitrogenous bodies—
Milk and cheese: G. E. Patrick, Washington, D. C.
Vegetable proteids: R. Harcourt, Guelph, Canada.
Potash:
Determination: T. Bragg, Auburn, Ala.
Availability: J. A. Bizzell, Ithaca, N. Y.
Soils: G. S. Fraps, College Station, Tex.
Dairy products: E. M. Bailey, New Haven, Conn.

Foods and feeding stuffs: E. V. McCollum, Madison, Wis.
Food adulteration:
Colors: W. E. Mathewson, New York, N. Y.
Saccharine products: C. P. Moat, Burlington, Vt.
Fruit products: A. W. Blair, Gainesville, Fla.
Wine: Julius Hortvet, St. Paul, Minn.
Beer: H. E. Barnard, Indianapolis, Ind.
Distilled liquors: R. E. Stallings, Atlanta, Ga.
Vinegar: R. W. Balcom, Nashville, Tenn.
Flavoring extracts: E. M. Chace, Washington, D. C.
Spices: A. F. Seeker, New York City.
Baking powder: Edmund Clark, Boston, Mass.
Meat and fish: J. A. Emery, Washington, D. C.
Fats and oils: T. J. Bryan, Chicago, Ill.
Dairy products: A. J. Patten, East Lansing, Mich.
Vegetables: J. P. Street, New Haven, Conn.
Condiments: H. E. Bishop, Indianapolis, Ind.
Cocoa: W. L. Dubois, Buffalo, N. Y.
Tea and coffee: M. E. Jaffa, Berkeley, Cal.
Preservatives: P. B. Dunbar, Washington, D. C.
Water in foods: P. F. Trowbridge, Columbia, Mo.
Organic and inorganic phosphorus in foods: H. S. Grindley, Urbana, Ill.
Cereal products: W. M. Allen, Raleigh, N. C.
Sugar: R. S. Hiltner, Denver, Colo.
Insecticides: H. R. Watkins, Washington, D. C.
Medicinal plants: C. H. La Wall, Philadelphia, Pa.; H. H. Rusby, New York, N. Y.
Water: W. W. Skinner, Washington, D. C.
Tannin: Burton Ray, West Raleigh, N. C.
Inorganic plant constituents: W. H. McIntire, State College, Pa.

SPECIAL COMMITTEES.

Amendments to the Constitution.

L. L. Van Slyke, Geneva, N. Y., chairman.
B. B. Ross, Auburn, Ala.
William Frear, State College, Pa.

Appropriation.

John Phillips Street, New Haven, Conn., chairman.
R. J. Davidson, Blacksburg, Va.
H. E. Barnard, Indianapolis, Ind.

Availability of Phosphoric Acid in Basic Slag.

W. F. Hand, Agricultural College, Miss., chairman.
C. B. Williams, West Raleigh, N. C.
C. G. Hopkins, Urbana, Ill.
H. D. Haskins, Amherst, Mass.
John S. Burd, Berkeley, Cal.

Compilation of By-laws of the Association.

W. D. Bigelow, Washington D. C., chairman.
C. L. Penny, Newark, Del.
R. N. Brackett, Clemson College, S. C.

Delegates to the United States Pharmacopœial Convention.

W. D. Bigelow, Washington, D. C.
A. L. Winton, Chicago, Ill.
E. H. Jenkins, New Haven, Conn.
Alternates: R. B. Fitz-Randolph, Trenton, N. J.; L. P. Brown, Nashville. Tenn.; C. E. Parker, Washington, D. C.

Food Standards.

William Frear, State College, Pa., chairman.
H. W. Wiley, Washington, D. C.
H. A. Weber, Columbus, Ohio.
M. A. Scovell, Lexington, Ky.
E. H. Jenkins, New Haven, Conn.

Presentation of the Question of Unification of Terms to the International Congress of Applied Chemistry.

R. J. Davidson, Blacksburg, Va., chairman.
C. G. Hopkins, Urbana, Ill.
W. D. Bigelow, Washington, D. C.
G. S. Fraps, College Station, Tex.
B. W. Kilgore, Raleigh, N. C.
H. J. Wheeler, Kingston, R. I.
J. T. Willard, Manhattan, Kans.

Recommendation of Referees and Revision of Methods.

(Figures in parentheses refer to year in which appointment expires.)

J. K. Haywood, chairman.

Subcommittee A: B. B. Ross (1914), J. P. Street (1912), *J. K. Haywood (1910), chairman, Bureau of Chemistry, Washington, D. C.*
Subcommittee B: E. M. Chace (1914), *F. W. Woll (1912), chairman, Agricultural Experiment Station, Madison, Wis.;* F. P. Veitch (1910).
Subcommittee C: C. D. Howard (1914), *A. L. Winton (1912), chairman, U. S. Food and Drug Inspection Laboratory, Chicago, Ill.;* L. M. Tolman (1910).

Standardization of Alcohol Tables.

L. M. Tolman, Washington, D. C., chairman.
M. E. Jaffa, Berkeley, Cal.
A. B. Adams, Washington, D. C.
R. J. Davidson, Blacksburg, Va.
H. E. Barnard, Indianapolis, Ind.

Testing of Chemical Reagents.

L. F. Kebler, Washington, D. C., chairman.
A. L. Winton, Chicago, Ill.
B. W. Kilgore, Raleigh, N. C.

Unification of Methods of Analysis of Fats and Oils.

L. M. Tolman, Washington, D. C., chairman.
P. H. Walker, Washington, D. C.
A. Lowenstein, Chicago, Ill.

CONSTITUTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

(1) This association shall be known as the Association of Official Agricultural Chemists of North America. The objects of the association shall be (1) to secure uniformity and accuracy in the methods, results, and modes of statement of analysis of fertilizers, soils, cattle food, dairy products, and other materials connected with agricultural industry; (2) to afford opportunity for the discussion of matters of interest to agricultural chemists.

(2) Analytical chemists connected with the United States Department of Agriculture, or with any state, provincial, or national agricultural experiment station or agricultural college, or with any state, provincial, or national institution or body in North America charged with official control of the materials named in section 1, shall alone be eligible to membership; and one such representative for each of these institutions or boards, when properly accredited, shall be entitled to enter motions or vote in the association. Only such chemists as are connected with institutions exercising official fertilizer control shall vote on questions involving methods of analyzing fertilizers. All persons eligible to membership shall become members ex officio and shall be allowed the privileges of membership at any meeting of the association after presenting proper credentials. All members of the association who lose their right to such membership by retiring from positions indicated as requisite for membership shall be entitled to become honorary members and to have all privileges of membership save the right to hold office and vote. All analytical chemists and others interested in the objects of the association may attend its meetings and take part in its discussions, but shall not be entitled to enter motions or vote.

(3) The officers of the association shall consist of a president, a vice-president, and a secretary, who shall also act as treasurer; and these officers, together with two other members to be elected by the association, shall constitute the executive committee. When any officer ceases to be a member by reason of withdrawing from a department or board whose members are eligible to membership, his office shall be considered vacant, and a successor may be appointed by the executive committee, to continue in office till the annual meeting next following.

(4) There shall be appointed by the executive committee, at the regular annual meeting, from among the members of the association, a referee and such associate referees for each of the subjects to be considered by the association as that committee may deem appropriate.

It shall be the duty of these referees to prepare and distribute samples and standard reagents to members of the association and others desiring the same, to furnish blanks for tabulating analyses, and to present at the annual meeting the results of work done, discussion thereof, and recommendations of methods to be followed.

(5) The special duties of the officers of the association shall be further defined, when necessary, by the executive committee.

(6) The annual meeting of this association shall be held at such place as shall be decided by the association, and at such time as shall be decided by the executive committee, and announced at least three months before the time of meeting.

(7) No changes shall be made in the methods of analysis used in official inspection, except by unanimous consent, until an opportunity shall have been given all official chemists having charge of the particular inspection affected to test the proposed changes.

(8) Special meetings shall be called by the executive committee when in its judgment it shall be necessary, or on the written request of five members; and at any meeting, regular or special, seven enrolled members entitled to vote shall constitute a quorum for the transaction of business.

(9) The executive committee will confer with the official boards represented with reference to the payment of expenses connected with the meetings and publication of the proceedings of the association.

(10) All proposed alterations or amendments to this constitution shall be referred to a select committee of three at a regular meeting, and after report from such committee may be adopted by the approval of two-thirds of the members present entitled to vote.

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